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A SYSTEMATIC STUDY OF *ONCOMELANIA HUPENSIS CHIUI* (GASTROPODA: HYDROBIIDAE)[1]

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ABSTRACT

The hydrobiid snail which Chiu (1961) reported to be the first intermediate host of *Paragonimus iloktsuenensis* in Taiwan (Formosa) was described as *Tricula chiui* by Habe & Miyazaki (1962). The snail, found in an isolated basin in northern Taiwan (A-li-lao area), was later shown to be susceptible to *Schistosoma japonicum* also (Chiu, 1965b, 1967). Anatomical data have now shown this snail to belong to the genus *Oncomelania*. It is closely allied to the subspecies of *O. hupensis*, as described for *O. h. formosana* (Davis, 1967) and for *O. h. nosophora* and *O. h. quadrasi* (Davis, unpublished), because: 1) the verge has a papilla and characteristic strip of cilia near the tip; 2) the seminal vesicle is knotted; 3) the spermathecal and sperm ducts arise from the right lateral edge of the bursa copulatrix near the anterior end as 2 separate tubes bound together in a common connective tissue sheath; 4) the oviduct coils over the seminal receptacle in a distinct manner; 5) the structure of the male and female gonad is similar; 6) the shell is distinctly of the *Oncomelania* type in spite of the obsolete varix.

Immunological studies using micro-Ouchterlony double diffusion tests with absorbed antiserum (anti- *O. h. formosana* foot muscle extract) indicated that *"Tricula chiui"* was more closely associated with populations of *O. h. formosana* than with other subspecies of *Oncomelania hupensis* because: 1) all antigen-antibody systems were homologous between them; 2) they had systems not present in or only partially identical with those occurring in the other subspecies of *O. hupensis*.

Polyacrylamide electrophoresis of proteins from foot muscle extract of *"T. chiui"* and several populations of *O. h. formosana* showed that the former had a densitometric profile of the separated protein components more similar to 1 population of *O. h. formosana* from N. E. Taiwan (I-lan county) than to any other.

Hybridization studies showed that *"T. chiui"* produced fertile hybrids with *O. h. formosana*.

From these collected data it appears most likely that the snail originally named *"Tricula chiui"* had its origin from stock giving rise to current *O. h. formosana* from I-lan county and that with subsequent isolation near the edge of the sea at A-li-lao, where tall mountains separate them from the I-lan region,

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this stock evolved characteristics justifying subspecific status, namely, 1) a much shortened shell, 2) significantly fewer gill filaments than other subspecies of *O. hupensis*, 3) shell with obsolete varix, 4) a longer pleuro-supraesophageal connective than found in *O. h. formosana*, 5) a larger supraesophageal ganglion, 6) a difference in frequency of cusp number on the various teeth of the radula. Accordingly the taxon is named *Oncomelania hupensis chiui*.

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INTRODUCTION

Chiu (1961) reported on the snail host of the lung fluke, *Paragonimus iloktsuenensis*, in Taiwan (Formosa). This undescribed snail, found by Chiu on the 24th of April, 1961 at A-li-lao village (Fig. 1), was subsequently described by Habe & Miyazaki (1962) as *Tricula chiui*. Further reports, mainly on the parasite, were made by Chiu (1962) and Miyazaki & Chiu (1962). Reports on the parasitological aspects involving the molluscan intermediate host were presented by Chiu (1965a, b, 1967), who showed that this snail was also capable of transmitting *Schistosoma japonicum*. The usual intermediate host of this blood Fluke is *Oncomelania*, a hydrobiid genus of the subfamily Pomatiopsinae.

Habe & Miyazaki (1962) stated that *Tricula chiui* differed from other species of *Tricula*, a genus in the Hydrobiidae, subfamily Triculinae, in having a broadly oval shell and that it was an ally to *"Katayama* (=*Oncomelania*) *formosana."*

In June 1963, Dr. Chiu found in the collection at the Mollusk Division, University of Michigan, an alcoholic lot of specimens collected by Dr. Robert E. Kuntz from A-li-lao, Taiwan. The lot was without specific designation and was recognized as *Tricula chiui*. I examined the anatomy and found a striking resemblance to the anatomy of the so-called species of *Oncomelania* (*O. formosana*, *O. nosophora*, *O. quadrasi* and *O. hupensis*) presently considered to be subspecies of *O. hupensis* (Davis, 1967). At that time studies were initiated to assess the relationship of *"Tricula chiui"* to the hydrobiid genera *Oncomelania* and *Tricula*. As a result, I have found this snail to be another subspecies of *Oncomelania hupensis*, i.e., *O. hupensis chiui*. The purpose of this paper is to present the results of studies involving the anatomy, hybridization, and some biophysical properties of this subspecies. The systematics, phylogeny and importance of parasitological relationships are discussed.

MATERIALS AND METHODS

Methods involving the varied techniques used are discussed in each of the separate sections dealing with different aspects of this study.

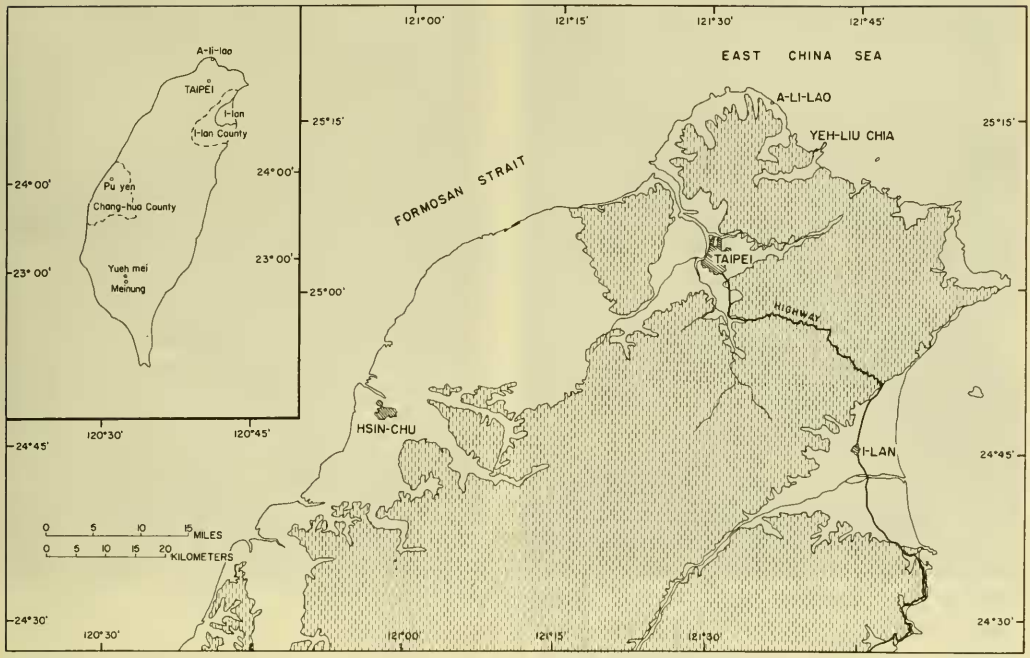


FIG. 1. Maps of Taiwan showing the localities for the populations studied in this paper. The type locality of *Oncomelania hupensis chiui* is at A-li-lao at the northern tip of the island. Shaded areas indicate mountainous regions.

Snails used in this study were *"Tricula chiui"* from the type locality at A-li-lao village in Taipei county, northern Taiwan, and, for comparison, various strains of *Oncomelania hupensis formosana* and other subspecies of *O. hupensis: O. h. quadrasi* came from Leyte, Philippines; *O. h. nosophora* was collected in Yamanashi Prefecture, Japan; progenitors of *O. h. hupensis* came from south China. In June, 1964, 2 populations of *O. h. formosana* were collected in I-lan county, north eastern Taiwan, and 1 population was collected from around Pu Yen village in Changhua county, in central western Taiwan (Fig. 1).

The collections were sent to the University of Michigan, Mollusk division, where they were placed in culture and used in electrophoretic and serological studies. Laboratory reared F_1 and F_2 generations, descended from the church population (Fig. 1) of I-lan snails, were also used for anatomical, electrophoretic and hybridization studies. In the immunological work, another strain of *Oncomelania hupensis formosana* was also used. These snails were descended from stocks (F_2 generation) that had been collected in Yueh Mei, southern Taiwan, by Dr. Kuntz in 1962. All anatomical studies were done at the 406th Medical Laboratory, Sagamihara City, Kanagawa Prefecture, Japan.

Habitat

From Figs. 1 and 2, it is evident that *Oncomelania hupensis chiui* is found on the coast of the northern tip of Taiwan. The habitat is exposed to winds off the sea laden with salt. The terrain is

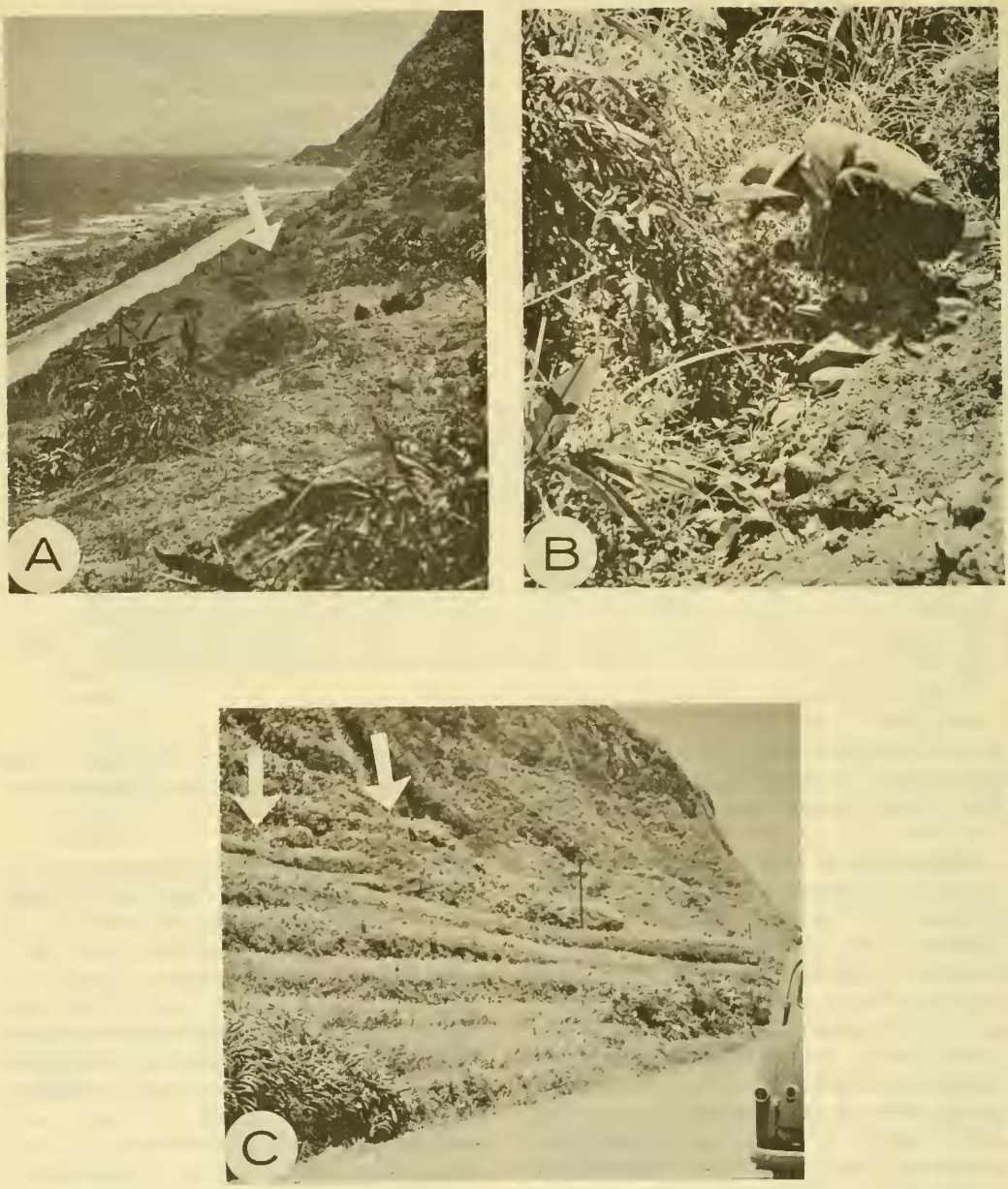


FIG. 2. Habitat of *Oncomelania hupensis chiui*.

A. General view showing the steep hilly nature of the habitat and its proximity to the sea.

B. Dr. Chiu peering into the small gully where water seeps downward in a shallow, gentle flow. The area is covered by dense vegetation.

C. The habitat is just above the terraced fields at the foot of the cliffs where the angle of the slope becomes quite steep.

rugged and the habitats are on steep slopes just where the slopes rise in abrupt cliffs (A, C, Fig. 2).

Snails were found in gullies eroded by channeled seepage and trickling water. The gullies (arrows, C, Fig. 2) are shallow, rocky and overgrown by dense brush. These amphibious snails are found submerged or out of water on rocks, leaves, sticks and small patches of soil. Dr Chiu is shown peering into one of the gullies (B, Fig. 2) which contains numerous snails of this subspecies.

This environment is in marked contrast to the usual habitats of the 4 previously known subspecies of *Oncomelania hupensis*, which inhabit the ditches around rice fields, irrigation canals and often the fields themselves, whereas the habitat of *O. h. chiui* is above the few terraced fields near the ocean and on steeper, more rugged terrain.

ANATOMY

1. Materials and Methods

Techniques used for dissections were described by Davis (1967). An additional technique was used in studying the nerves associated with the visceral ganglion. The uncoiled living snail was stained with Evan's blue dye (1:1000 aqueous) for 5-10 minutes. The result was a blue-stained epithelium with nerve tracts standing out white beneath the thin, ventral epithelium enclosing the mantle cavity.

Throughout the presentation of anatomical data, comparisons are made with *Oncomelania hupensis formosana* and *Pomatiopsis lapidaria* as given by Davis (1967). The latter snail serves for comparison with a genus of the Pomatiopsinae that is closely related to *Oncomelania* but qualitatively different in many aspects of anatomy. The orientation of organs and systems in the figures of this paper corresponds to that of the earlier study (Davis, 1967). All illustrations of anatomy were made by the author.

2. Shell

Habe & Miyazaki (1962) described the shell as "small, measuring about 4.5 mm in height, broadly ovate, rather solid, colored yellowish brown to brown. Nuclear whorls about two in number, smooth and polished, but usually eroded in the adult specimens. Post-nuclear whorls about 5, moderately inflated. Suture well impressed. Surface sculptured with numerous growth lines. Body whorl large and rounded, taking about two-thirds of the height of shell. Periphery obtusely angular in the young specimens, but well rounded in the fully grown specimens. Aperture rather large, roundly ovate, somewhat oblique, and slightly expanded. Peristome continuous, adnate at the parietal wall and colored dark brown at the edge. Outer lip short, arcuate and the columella short and weakly arched. Umbilical chink narrowed by the dilation of the columellar lip."

They figured the type and a paratype. Their material had 4.5-5.0 whorls, an average length and width of 4.46 and 2.73 mm respectively, and a ratio of length to width (L/W) of 1.63. Characteristic for these field specimens was the lack of any varix, i.e., the outer lip was thin and sharp. It was noted, however, that the umbilicus was more pronounced that the mere "chink" typical for several species of genuine *Tricula*.

In Fig. 3 are photographs of 3 topotypes collected by Robert E. Kuntz in 1961. The top 2 whorls in the population of 50 adults in that collection were without fail eroded and shells showed signs of weathering. Shells from that and later collections averaged 4.5-5 whorls, with an average length and width of 4.34 mm and 2.64 mm respectively. The L/W ratio was 1.64.

Snails were maintained in the laboratory as prescribed by Davis (1967) and van der Schalie & Davis (1968). Young were reared according to methods of van der Schalie & Davis (1965). Three laboratory reared snails are shown in

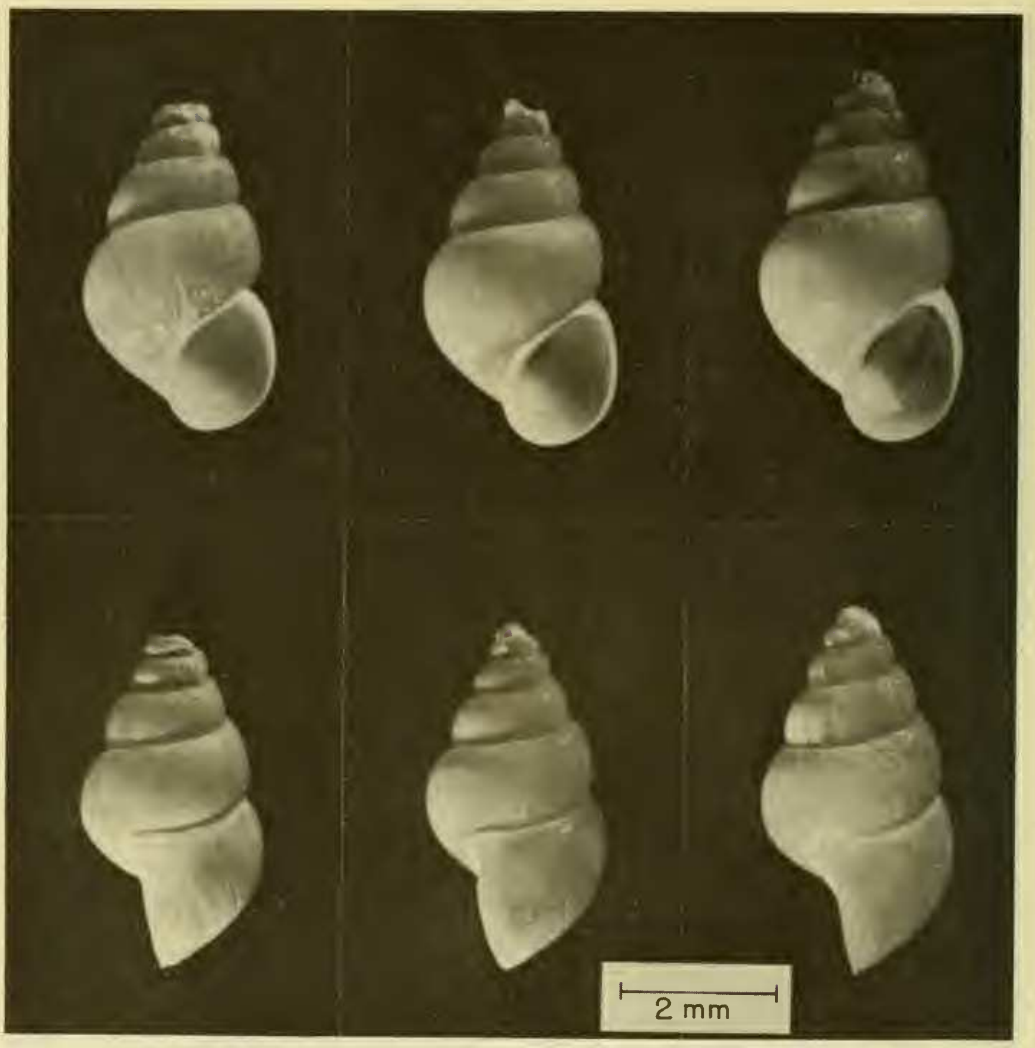


FIG. 3. Field collected *Oncomelania hupensis chiui*. Note sharp outer lip without varix and the eroded apex.

Fig. 4. Statistics pertaining to the shell are given in Table 1.

In Petri dish cultures snails reached adult size in 8 weeks, and did not grow any further when maintained singly beyond 9 weeks. There was a clear sexual dimorphism in whorl count and length. Males attained 6.0 whorls and averaged 4.36 mm in length while females with 6.5 whorls were larger, averaging 4.73 mm.

Perfect shells with complete spire indeed look like members of the *Oncomelania* complex. In Table 2 are given statistics on shell measurements for F_1 laboratory reared *O. h. formosana*, *O. h. chiui* and *O. h. quadrasi*. The data for *O. h. formosana* were taken from Davis (1967). All snails were reared in the same manner. Except for being wider at the base, the shell of *O. h. chiui* more closely resembles that of *O. h. quadrasi*.

Abbott (1948) says of the latter sub-

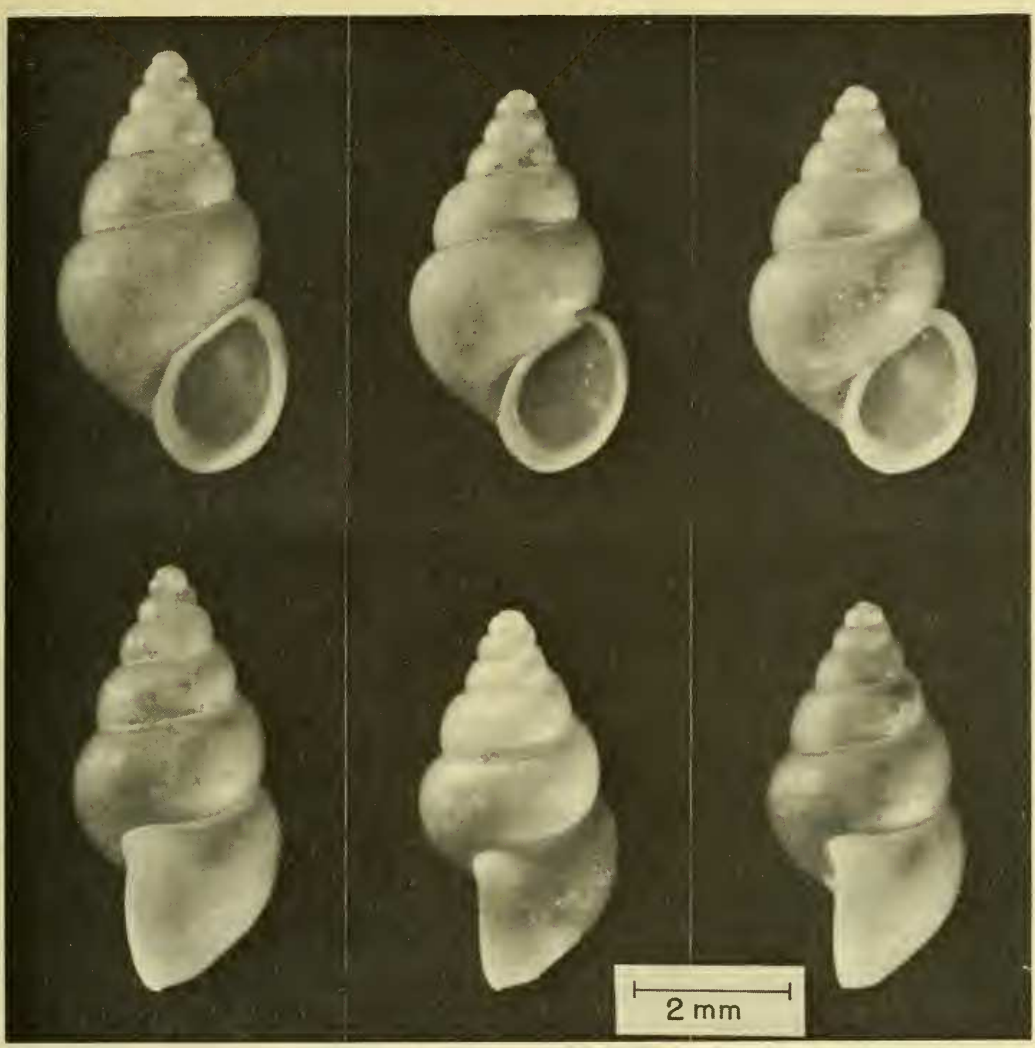


FIG. 4. First generation laboratory reared *Oncomelania hupensis chiui*. Note the perfect apices and the very slight thickening at the outer lip.

species that the adult shell "is from 3-5 mm in length with 6 to 7 whorls." He further states that the varix is generally only slightly developed. In the populations of *O. h. quadrasi* I collected in the Philippines from Oriental Mindoro, Leyte and South Luzon, the character of the varix varied greatly from well developed to nearly absent. In the laboratory reared populations the varix was nearly always discernible in adults, but slightly less pronounced than that shown by Davis (1967) for *O. h. formosana*.

In laboratory reared *Oncomelania hupensis chiui*, the varix was faint. The lip was thickened, as shown in Fig. 4, but did not produce the marked outfolding of shell moderately developed in *O. h. quadrasi* or pronounced in *O. h. formosana*. The point to be made is that under optimal conditions the lip was not simple, thin and sharp as in the field snails, but had a faint varix.

TABLE 1. Various parameters measured on 9 adult shells of *Oncomelania hupensis chiui* reared in the laboratory

Feature measured (mm) or counted	Sex	Statistics		
		$\bar{X}$	S	Se
Number of whorls	♂	6.0	-	-
	♀	6.5	-	-
		mm	mm	mm
Length shell	♂	4.36	0.09	0.03
	♀	4.73	0.15	0.05
Width shell	♂	2.47	0.07	0.02
	♀	2.74	0.12	0.04
Length parietal callus		0.94	0.02	0.006
Length aperture		1.89	0.12	0.04
Apical whorl:				
Width		0.35	0.02 (t.v.)	
Width of tip		0.14	0.02 (t.v.)	

$\bar{X}$ = Mean
S = Standard deviation
Se = Standard error of the mean
t.v. = total variation

TABLE 2. Comparison of adult shell[2] dimensions of 3 subspecies of *Oncomelania hupensis*

Shell feature measured (mm) or counted	Taxa		
	chiui (9)	*quadrasi* (25)	*formosana* (25)
Greatest number of whorls	6.5	6.5	7.0 - 7.5
Length of shell	4.73	4.79	6.30
Width of shell	2.74	2.62	3.00
Ratio L/W	1.73	1.83	2.10
Length aperture	1.89	2.04	2.40
Length parietal callus	0.94	0.94	1.08
Width apical whorl	0.35	0.35	0.34
Width of tip of apical whorl	0.14	0.14	0.12

[2] Shells were picked at random from snails having the greatest whorl count and in which varix formation (*O. h. quadrasi, O. h. formosana*) or a thickened outer lip (*O. h. chiui*) were observed.

As discussed and illustrated by Davis (1967) there is a sinuation of the outer lip in *Oncomelania hupensis formosana*. In field *O. h. chiui* (Fig. 3) the lip is straight or shows only the slightest sinuation. The laboratory reared snails Fig. 4) showed the more typical sinuation found in *O. h. formosana* and *O. h. quadrasi*.

On the basis of shell, it is concluded that the snail under consideration belongs in the *Oncomelania* complex. The

only difference is the very slight development of varix.

3. External Morphology and Topography

External features of the head area and mode of progression clearly indicate that *Oncomelania hupensis chiui* is a member of the Hydrobiidae, subfamily Pomatiopsinae.

The pedal crease is evident in the step-like progression of the animal. The suprapedal fold is present, as is the omniphoric groove (previously discussed by Davis, 1967). The head area is evenly dusted with grey pigment.

With the animal moving about under water the extended tentacles were 1.00-1.09 mm long in the adults, measurements comparing well enough with the length range of 0.96-1.20 mm for *Oncomelania hupensis formosana* (Davis, 1967). The tentacles bear eyes in pronounced swellings at their outer bases. The mid-lateral border of the eyes is encircled by white granules which, in a few cases, extend in front of the eyes and a very short way out on the tentacles. These "eyebrows" are white, not yellow as in *O. h. quadrasi* or pale yellow to white-yellow as in *O. h. formosana.*

Sexual dimorphism is evident in the pigment pattern of the animal, as was the case in *Oncomelania hupensis formosana.* In the apical body whorls of males, the dorsal surface of the stomach and digestive gland is covered with a uniform dark pigment (Pi; Fig. 7A). About 20% of the laboratory reared female snails had only a slight amount of pigment, in a narrow strip 0.063 mm wide near the apex. In field collected females pigment was frequently found lightly dusting the stomach. About 2/3 of the digestive gland was devoid of pigment but at the tip a narrow strip often traversed the mid-dorsal region of the gland.

The arrangement of organs is exactly as described for *Oncomelania hupensis formosana* (Davis, 1967). The relative sizes of digestive gland and total body length for *O. h. chiui*, *O. h. formosana* and *Pomatiopsis lapidaria* are shown in Table 3. *O. h. chiui* is clearly an order of magnitude smaller than *O. h. formosana.*

4. Mantle Cavity

In field snails there were 32-36 ctenidial filaments (without correlation to sex). Laboratory reared adult males had 26+ 5 gill filaments while females had 32+ 4. These data differ considerably from those of Habe & Miyazaki (1962) who state that "gill lamellae moderately developed, about 20 in number."

The length of the row of gill filaments is similar in the 2 subspecies of *Oncomelania hupensis* and distinctly greater than in *Pomatiopsis lapidaria* (Table 3).

The relationship of the osphradium to the gills and to the anterior end of the mantle cavity is shown in Fig. 10. The osphradial ganglion (Og) is clearly discernible within the osphradial pit (Opi). The positional relationship between osphradium and ctenidium is the same in both *Oncomelania hupensis formosana* and *O. h. chiui*, although the osphradium is somewhat larger in the latter (Table 3).

Organs within the mantle cavity are the same as those discussed in detail by Davis (1967).

5. Female Reproduction System (Figs. 5, 6)

The female reproductive system is similar in structure and position of organs to that in *Oncomelania hupensis formosana.* The uncoiled female is shown in Fig. 5.

The oviduct (Ov) is shown broken, due to uncoiling the body. The gonopericardial duct (not figured) was found leading into the kidney tissue (Ki) from the dorsal side of the oviduct where it turns medially.

Gonad. The gonad (Go; Figs. 5; 6A, D-F) is multibranched (3-5 branches). It is distinctly shorter than that of *Oncomelania hupensis formosana* and *Pomatiopsis lapidaria*, but has

TABLE 3. Comparison of organ size (mm), in forms of *Oncomelania hupensis* and *Pomatiopis* exclusive of the nervous system[3]

Organ	Dimension	Sex	Taxa		
			O. h. chiui mm	*O. h. formosana* mm	*P. lapidaria* mm
1. Tentacles	L	-	1.00-1.09	0.96-1.20	0.60-0.90
2. Digestive gland	L	♀	2.66-2.90	3.23*	4.78*
	W (at stomach)	♀	0.73-0.89	1.40*	1.07*
3. Total body	L	♀	7.4*	8.9*	10.6*
4. Gills	Number	♂	26 ± 5	46 ± 4	22 ± 2
		♀	32 ± 4		25 ± 3
	L	-	1.94 ± 0.24	1.91*	1.57*
5. Osphradium	L	-	0.63; S, 0.22	0.50 ± 0.09	0.59 ± 0.12
6. Buccal mass	L	-	0.720-0.780	0.720-0.860	0.850-1.080
7. Reproductive system		♀			
1) Gonad	L		0.79-1.23	1.12-1.61	1.10-1.61
2) Bursa copulatrix	L		0.73-0.92	0.74-0.94	0.58-0.86
	W		0.34-0.48	0.33-0.43	0.45-0.51
3) Seminal receptacle:					
Duct	L		0.24†	0.14-0.19	0.12-0.25
	W		0.03†	0.05†	0.06-0.15
Swollen portion	L		0.21†	0.17-0.31	0.20-0.24
	W		0.15†	0.12-0.24	0.17-0.24
4) Pallial oviduct	L		3.14 ± 0.20	3.36-4.00	4.30-5.00
	W (greatest)		0.60 ± 0.12	0.60*	0.72*
1) Prostate	L	♂	1.69-2.18	2.20-2.25	1.68-1.75
	W		0.68-0.73	0.62-0.72	0.70
2) Verge	L		2.18-2.66	3.36 ± 0.12	2.60-3.40
	W (base)		0.68-0.85	0.63*	0.63*

L = length
W = width
S = standard deviation
± = gives total variation
* = measurement from 1 adult individual
† = variability not encountered in measuring 6 individuals

[3] Measurements were taken from 6-12 adult individuals unless otherwise indicated. The total range is given without a mean value when only 6-7 measurements were made.

the same structure as *O. h. formosana*. Although *O. h. formosana* and *P. lapidaria* have the same size ovary, their structure is quite distinct and different (Davis, 1967).

Bursa copulatrix. The bursa (Bu) is shown from the ventral aspect in Figs. 5A; 6A, B. Its length and width in the 3 taxa are compared in Table 3. Size and shape in the 2 subspecies of *Oncomelania* are comparable.

The sperm duct (Sdu) and spermathecal

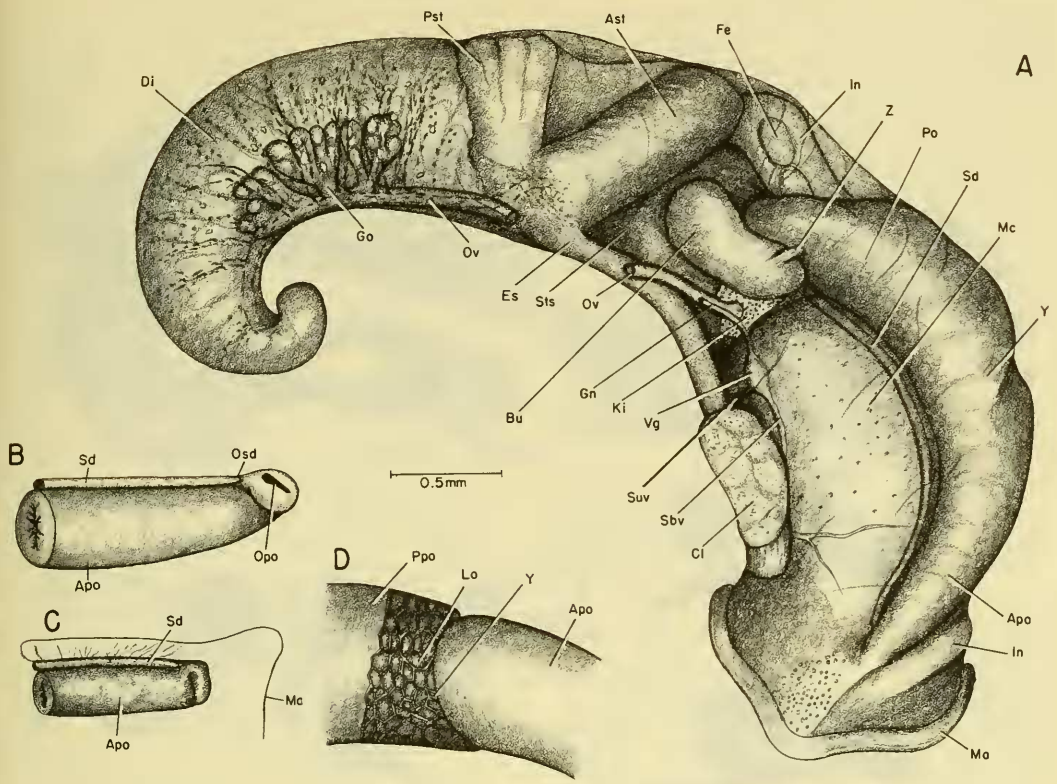


FIG. 5. Female reproductive system of *Oncomelania hupensis chiui*.

A. Uncoiled female showing the ventral or columellar side of the body. The kidney tissue was removed, in part, to reveal the entire bursa copulatrix (Bu); only the tissue encircling the oviduct near the end of the mantle cavity was not removed.

B. Anterior portion of the pallial oviduct (Apo) and spermathecal duct. The anterior end of the pallial oviduct has been bent towards the viewer to expose the opening.

C. Anterior portion of the pallial oviduct and spermathecal duct as normally viewed with the cut mantle folded back to expose this region.

D. Region (Y) where the posterior and anterior portions of the pallial oviduct join. The network of connective tissue chambers seen beneath the epithelium of the posterior section is partially indicated.

Apo	anterior section of the pollial oviduct
Ast	anterior chamber of the stomach
Bu	bursa copulatrix
Cl	columellar muscle
Di	disgestive gland
Es	esophagus
Fe	fecal pellet
Go	gonad
Gn	gonadal nerve
In	intestine
Ki	kidney
La	a section of the lattice-work of connective tissue enclosing the glandular units found throughout the posterior portion of the pallial oviduct
Ma	mantle edge
Mc	epithelium covering the mantle cavity
Opo	opening of the pallial oviduct
Osd	opening of the spermathecal duct
Ov	oviduct
Po	pallial oviduct
Ppo	posterior portion of the pallial oviduct
Pst	posterior chamber of the stomach
Sbv	subvisceral connective
Sd	spermathecal duct
Sts	style sac
Suv	supravisceral connective
Vg	visceral ganglion
y	point where the anterior and posterior sections of the pallial oviduct join
z	ducts leaving the bursa copulatrix

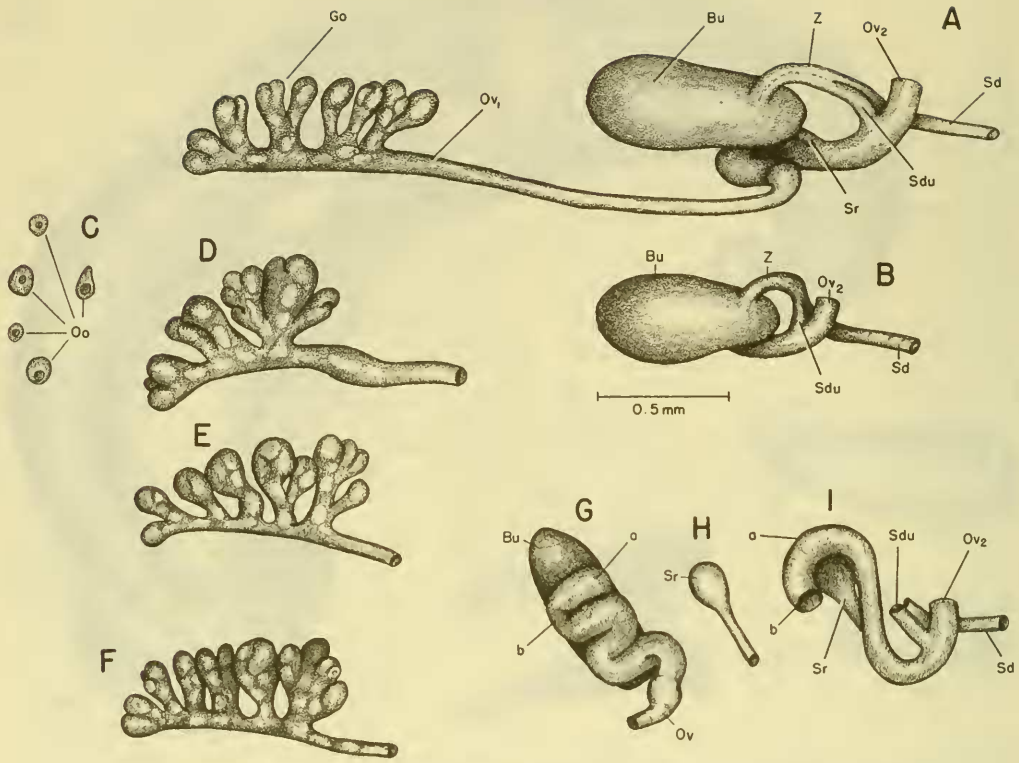


FIG. 6. Female reproductive system of *Oncomelania hupensis chiui*.

A. Arrangement of ducts associated with the gonad and bursa copulatrix, before the oviduct enters the pallial oviduct. Dashed line shows that the tubes (spermathecal and sperm ducts) leaving the bursa are separate but enclosed in a common connective tissue sheath.

B. Variation in shape of bursa copulatrix and the ducts leaving the bursa, as seen in gross dissection.

C. Oocytes of various sizes and shapes as dissected from the gonad.

D–F. Variation in branching and structure of the gonad.

G. The bursa rotated to show the dorsal surface with coiled oviduct. The coils occlude the seminal receptacle.

H. Seminal receptacle.

I. A portion of the coiled oviduct removed to show relationship of seminal receptacle, oviduct and spermathecal duct.

a	tip of coil of the oviduct dorsal to the bursa copulatrix corresponding to the equivalent points in Figs. G and I
b	point shown if Figs. G and I, cut in Fig. I to reveal the seminal receptacle (Sr)
Bu	bursa copulatrix
Go	gonad
Oo	oocytes
Ov_1	oviduct, from gonad to the sperm duct (Sdu)
Ov_2	oviduct, from sperm duct to entry into pallial oviduct
Sd	spermathecal duct
Sdu	sperm duct
Sr	seminal receptacle
z	ducts leaving the bursa copulatrix in common sheath

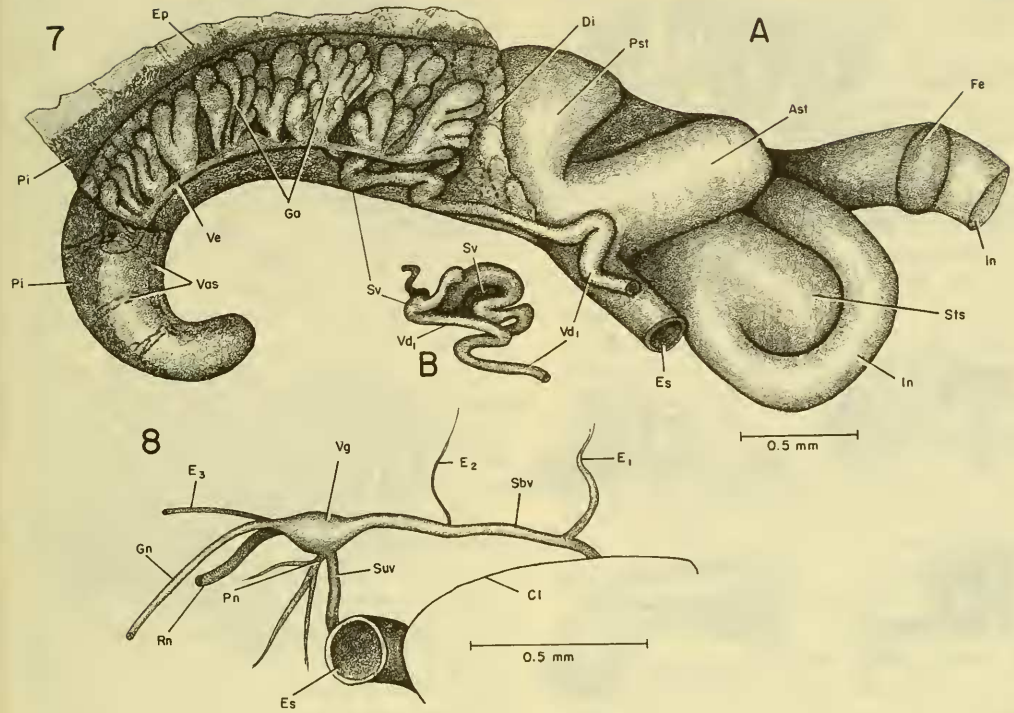


FIG. 7. Male reproductive system of *Oncomelania hupensis chiui*.

A. Uncoiled mid- and posterior body of a male, ventral view. The digestive gland abuts on the stomach. The connective and kidney tissues were removed to show the exterior ventral structure of the stomach.

B. Coiled section of the vas deferens (seminal vesicle) which is covered in Fig. A by the anterior section of the gonad.

FIG. 8. The visceral ganglion complex showing an unusual variation in the position where the pericardial nerve arises. The nerve (Pn) usually arises from the supravisceral connective (Suv) slightly removed from the visceral ganglion (Vg) as shown in Fig. 12.

Ast	anterior chamber of the stomach	Pi	pigment
Cl	columellar muscle	Pn	pericardial nerve
Di	digestive gland	Pst	posterior chamber of the stomach
E_1	external mantle cavity nerve 1	Rn	renal nerve
E_2	external mantle cavity nerve 2	Sbv	subvisceral connective
E_3	external mantle cavity nerve 3	Sts	style sac
Es	esophagus	Suv	supravisceral connective
Fe	fecal pellet	Sv	seminal vesicle
Ep	epithelium	Vd_1	posterior section of vas deferens
Gn	gonadal nerve	Vas	vascular elements and visceral artery
Go	gonad	Ve	vas efferens
In	intestine	Vg	visceral ganglion

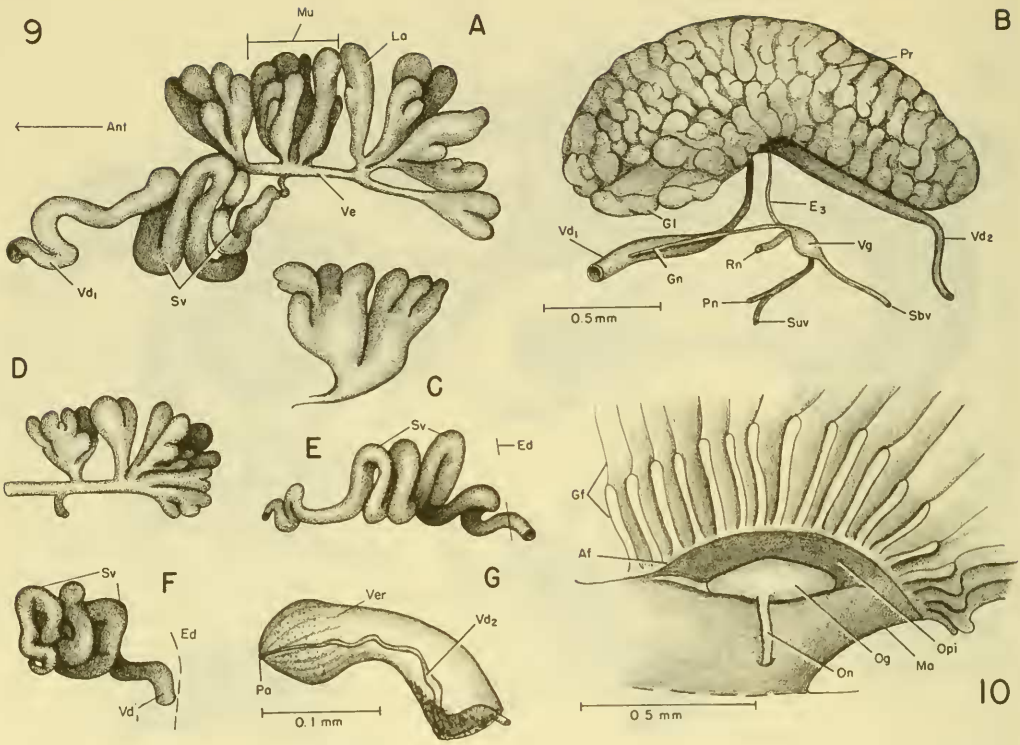


FIG. 9. Male reproductive system of *Oncomelania hupensis chiui*. Figs. A-F are at the same scale.

A. Dorsal aspect of the gonad with the anterior multibranched units (Mu) removed. The seminal vesicle, and the point of connection with the vas efferns, are shown. In this specimen this connection was in an unusual position, being slightly posterior to mid-gonad. Generally, one observes more individual lobes (Lo) and how they arise and branch, from the dorsal aspect than from the ventral (compare with Figs. 7A and 9D).

B. Ventral view of prostate showing relationship to anterior and posterior vas deferens and visceral ganglionic complex.

C. A single lobe from a multibranched testicular unit viewed posteriorly. It represents the width of the gonad.

D. Ventral aspect of several multibranched units at the anterior end of the gonad from a different specimen than the one used for Fig. 9A.

E-F. Variation in coiling of the seminal vesicle. Dashed line represents anterior end of the digestive gland.

G. The verge.

FIG. 10. Relationship of ctenidium and osphradium. The posterior gill filaments are not shown.

Af	afferent vessel	Gn	gonadal nerve
Ant	anterior direction	Lo	individual testicular lobe from a multi-branched gonodal unit
Ed	anterior end of digestive gland	Ma	anterior mantle, edge of the reflected mantle
E_3	external mantle cavity nerve 3	Mu	one multibranched gonodal unit
Gf	Gill filament		
Gl	individual glandular unit of the prostate		

duct (Sd) arise from the bursa just as they do in *Oncomelania hupensis formosana*, bound together by connective tissue so as to appear as 1 tube (z; Figs. 5; 6A, B). The dotted line in Fig. 6A represents the actual internal division of those tubes. As in *O. h. formosana*, these ducts are overgrown by tissue of the pallial oviduct (Po; Fig. 5A). The area just anterior to the bursa (Fig. 5A) is further occluded by kidney tissue (Ki) and connective tissue. When the overlying tissues are removed, the relationship of the tubes becomes clear (Fig. 6A, B). The sperm duct (Sdu) and the spermathecal duct (Sd) diverge, the former bending to join the oviduct (Ov_2, Fig. 6A, B), the latter passing medially and dorsal to the oviduct just where the oviduct enters the pallial oviduct to continue along the mantel cavity.

Seminal receptacle. When the bursa copulatrix (Bu) as shown in Fig. 6A is turned over 180° (Fig. 6G) one observes the dorsal convoluted section of the oviduct. The coils are pressed against the bursa copulatrix hiding from view the seminal receptacle situated between them. Removal of part of the coil (Fig. 6I) shows that the oviduct coils around the seminal receptacle (Sr). The arrangement of this coiled section of oviduct and the manner in which it encircles the seminal receptacle are exactly as found in *O. h. formosana*.

The seminal receptacle is similar in shape to that of *O. h. formosana* (Sr; Fig. 6H). It is compared with this organ in the other taxa in Table 3. The duct from the swollen portion of the receptacle to the oviduct is noticeably very narrow (0.03 mm wide). As shown in Fig. 6A, the duct (Sr) enters the oviduct close to the opening of the sperm duct (Sdu). Most frequently this point is occluded by the anterior edge of the bursa (Fig. 6B).

The swollen receptacle is bound to the bursa by distinct connective tissue in which are imbedded numerous white granules.

Pallial oviduct. The pallial oviduct is a huge organ which overlies the organs at the mid-body region, the posterior end of the mantle cavity and the anterior mantel cavity (Po; Fig. 5A). The organ is clearly separable into 2 sections when viewed at 16X-40X under direct illumination; a grey-white posterior section (Po) and a white anterior section (Apo). The dividing point is shown in Fig. 5A by the pronounced dip (at y) which occurs at the outer curvature about mid gland. The sections differ in texture; the posterior section appears more glandular, the epithelium is stretched by loose swollen glandular pockets bounded by a definite honeycomb-like framework of connective tissue (La; Fig. 5D). The anterior section is more slender and composed of solid white tissue lacking the glandular macroscopic units.

The length and greatest width of the organ are compared with that found in other species in Table 3. It is not as long as in *Oncomelania hupensis formosana*, but is as wide at the posterior end.

Cutting the mantle between the columellar muscle (Cl) and spermathecal duct (Sd; snail oriented as in Fig 5A) and folding back the mantle, exposes the anterior end of the pallial oviduct and

---

Og	osphradial ganglion
On	osphradial nerve
Opi	osphradial pit
Pa	papilla
Pn	pericardial nerve
Pr	prostate
Rn	renal nerve
Sbv	subvisceral connective
Suv	supravisceral connective
Sv	seminal vesicle
Vd_1	vas deferens from gonad to prostate
Vd_2	pallial vas deferens; from prostate to tip of verge
Ve	vas efferens
Ver	verge
Vg	visceral ganglion

spermathecal duct (Fig. 5C). As shown in that figure, the thickened lip at the anterior end of the pallial oviduct (Apo) presses over its opening. When the end of the organ is pulled upward and bent slightly toward the viewer the opening is seen (Opo; Fig. 5B).

The spermathecal duct terminates, as shown (Osd; Fig. 5B), before reaching the thickened lips of the terminal pallial oviduct. The relationship of these organs to each other and the mantle cavity is the same in *Oncomelania hupensis formosana* and is more fully discussed by Davis (1967) for that taxon.

6. Male Reproductive System (Figs. 7, 9)

This system corresponds in organ structure and position to that in *Oncomelania hupensis formosana* (Davis, 1967).

Gonad. The posterior section of the body tube is shown uncoiled in Fig. 7A. The digestive gland (Di) abuts on the posterior section of the stomach (Pst). A strip of epithelium (Ep) is pulled back to clearly show the gonad (Go) which lies just beneath the epithelium.

From 7-9 multibranched units (Mu; Fig. 9A) arise from a slender vas efferens (Ve). The vas deferens (Vd_1) usually arises from the anterior 1/3 of the vas efferens. The drawings in Figs. 7A, 9A and 9D were made using different specimens. The points where the lobes (Lo) arise from the vas efferens or from a common duct arising from the vas efferens are generally more clearly seen from the dorsal aspect (Fig. 9A). In ventral view (Figs. 7A and 9D) the basal portions of a greater number of lobes are obscure. Figs. 9A, D show the relationship of the lobes in the multibranched units and how the units arise from the vas efferens. Fig. 9C shows the width of the gonad as composed of 2 lobes.

Seminal vesicle. The vas deferens becomes a spherical knotted mass of tubes (Sv) soon after leaving the vas efferens (Figs. 7B, 9A). This confusing knot of tubes is called the seminal vesicle. As viewed and shown in Fig. 7A, the seminal vesicle is hidden beneath the anterior 1/3 of the gonad. Only the lateral edge is showing where the vas deferens (Vd_1) leaves the coil and runs toward the stomach. With the gonadal lobes removed, the seminal vesicle is exposed (Fig. 7B). Variations in coiling are shown in Fig. 9A, E, F. The dashed line (Ed) in the figures indicates the anterior end of the digestive gland.

Prostate. The prostate (Fig. 9B) occupies the same position as the pallial oviduct in the female but is not as long. It overlaps the posterior end of the mantle cavity.

The glandular nature of the ventral prostate surface is shown in Fig. 9B. The length and greatest width (posterior end) of the prostate are compared with that of the other taxa in Table 3. Greater detail of the anatomy of the prostate and how the posterior vas deferens (Vd_1) and pallial vas deferens (Vd_2) connect with it, is given by Davis (1967) for *Oncomelania hupensis formosana*, which has the same prostate anatomy.

Verge. The verge (penis) is shown in Fig. 9G. It does not differ from that of the other subspecies of *Oncomelania*. The anterior end is muscularly thickened. In this area longitudinal muscle strands are evident. There is a protrudable papilla at the tip of the verge which is otherwise blunt and flattened.

The verge is ciliated exactly as described for *Oncomelania hupensis formosana* (Davis, 1967). Likewise, the glandular units correspond. The length of verge and width at the base are compared for the 3 snails in Table 3.

7. Muscular system

The only portion of the muscular system illustrated or discussed here pertains to the exterior buccal mass (Fig. 11A). The musculature, as a whole, is that described for *Oncomelania hupensis formosana* and is similarly labeled (Davis, 1967). Not shown in Fig. 11A are suspensors of the buccal

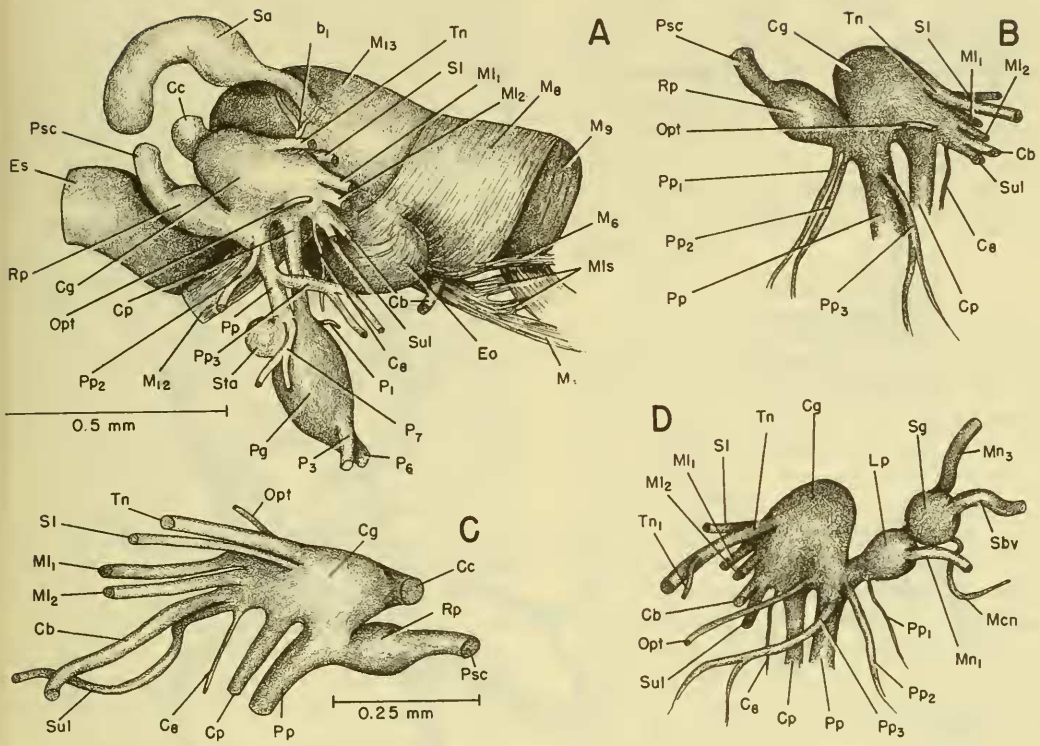


FIG. 11. Nervous and muscular system of *Oncomelania hupensis chiui*.

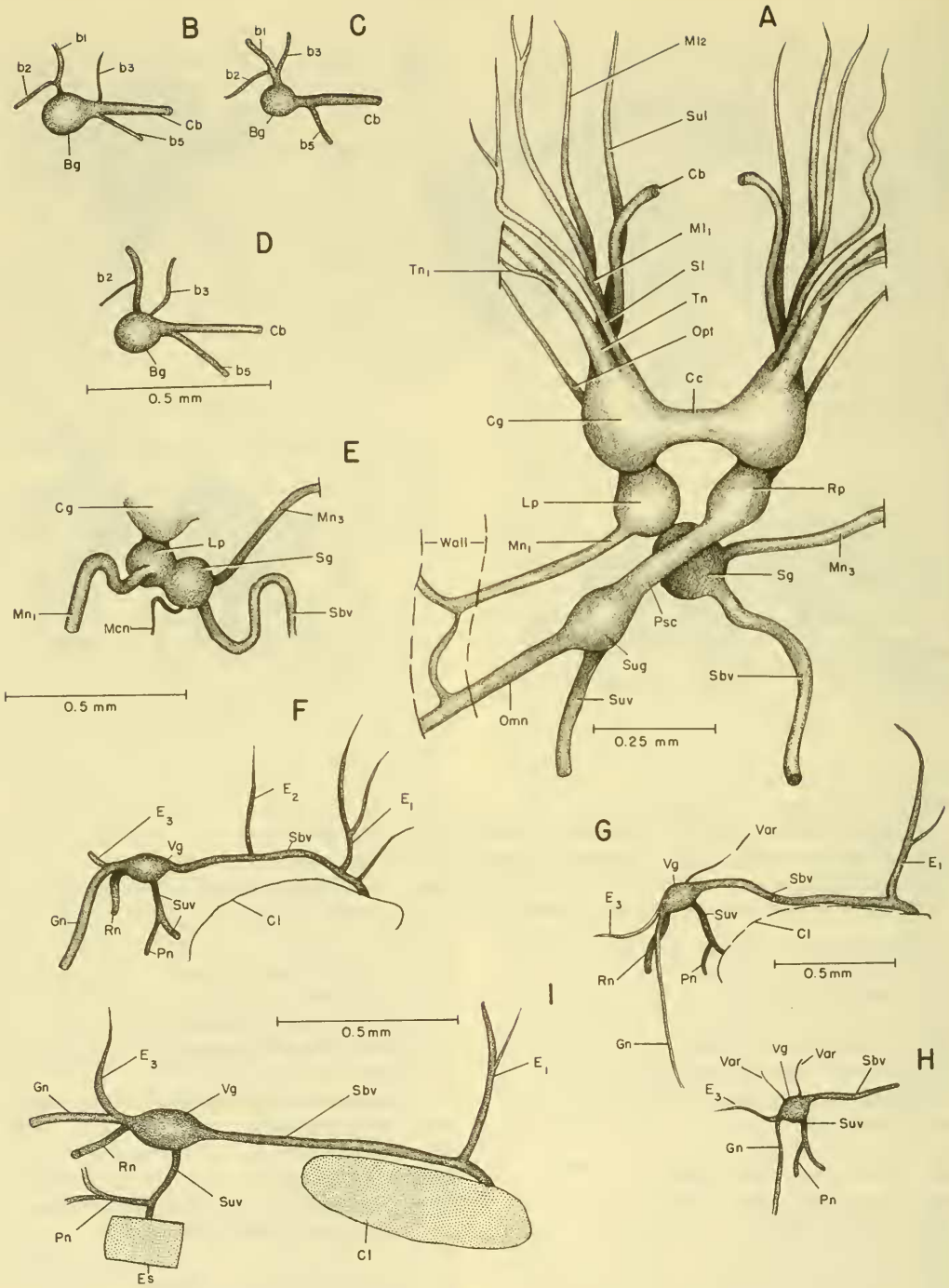
A. Right lateral aspect of buccal mass, cerebral and pedal ganglia.

B. Right cerebral and pleural ganglia showing variation in emergent nerves.

C. Medial aspect of right cerebral ganglion showing where the nerves arise.

D. Left cerebral ganglion, pleural ganglion, and subesophageal ganglion.

b_1	dorsal buccal nerve
Cb	cerebro-buccal connective
Cc	cerebral commissure
Cg	cerebral ganglion
Cp	cerebro-pedal connective
C_8	cerebro-tensor nerve
Eo	external odontophore membrane
Es	esophagus
Lp	left pleural ganglion
M_5	buccal protractor muscle
M_6	preventral protractor
M_8	anterior jugalis
M_9	buccal constrictor
M_{12}	buccal retractor
M_{13}	membranous jugalis
Mcn	midcolumellar nerve
Ml_1	median labial nerve 1
Ml_2	median labial nerve 2
Mls	medio-lateral slips of buccal protractor
Mn_1	mantle nerve 1
Mn_3	mantle nerve 3
Opt	optic nerve
P_1	lateral retractor nerve
P_3	major lateral nerve
P_6	metapodial connective
P_7	dorso-lateral pedal nerve
Pg	pedal ganglion
Pp	pleuro-pedal connective
Pp_1	lateral nerve 1
Pp_2	penial nerve
Pp_3	lateral nerve 3
Psc	pleuro-supraesophageal connective
Rp	right pleural ganglion
Sa	salivary gland
Sbv	subvisceral connective
Sg	subesophageal ganglion
Sl	supralabial nerve
Sta	statocyst
Sul	sublabial nerve
Tn	tentacular nerve
Tn_1	branch, tentacular nerve


A
Ml2
Sul
Cb
Ml1
Sl
Tn
Opt
Cc
Tn1
Cg
Lp
Rp
Wall
Mn1
Mn3
Sg
Psc
Sug
Sbv
Suv
Omn
0.25 mm
B
b1
b2
b3
Cb
b5
Bg
C
b1
b3
b2
Bg
Cb
b5
D
b2
b3
Cb
Bg
b5
0.5 mm
E
Cg
Mn3
Lp
Sg
Mn1
Mcn
Sbv
0.5 mm
F
E2
E1
E3
Vg
Sbv
Gn
Rn
Suv
Cl
Pn
G
Var
Vg
E1
Sbv
E3
Suv
Cl
0.5mm
Rn
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I
0.5mm
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Cl
Es
H
Var
Vg
Var
Sbv
E3
Suv
Gn
Pn

mass and the preventral dilators. Both groups are present; the former running between the muscles M_8, M_9 and the roof of the cephalic haemocoel, the latter from M_9 to the lateral floor of the cephalic haemocoel.

The well defined medio-lateral slips (Mls) of the buccal protractor (M_5) differed slightly from their counterpart in *O. h. formosana*. In the latter subspecies the buccal protractor has 2 slips which are either united in a single sheet or, more frequently, split. When split, one slip originates on the rostral retractor, the other on the anterior ventro-lateral rostral wall. In *O. h. chiui* the most common arrangement consists of 2 slips as described for the above subspecies. However, 3 slips are frequently encountered, in which case 2 distinct bands instead of 1 (medio-lateral slips of the buccal protractor, Mls, Fig. 11) originate from the rostral retractor or the oral sphincter (anterior, ventral rostral region) and insert on the main band of the buccal protractor (M_5, Fig. 11).

8. Nervous System (Figs. 5, 8-13)

The nervous system was dissected with the following in mind: 1) to present all nerves arising from each ganglion as they actually appeared; 2) define limits of variation in these main nerves in terms of position and number; 3) compare the above with *Oncomelania hupensis formosana*. Orientation in the illustrations and terminology are the same as presented for *O. h. formosana* (Davis, 1967).

A. Cerebral complex

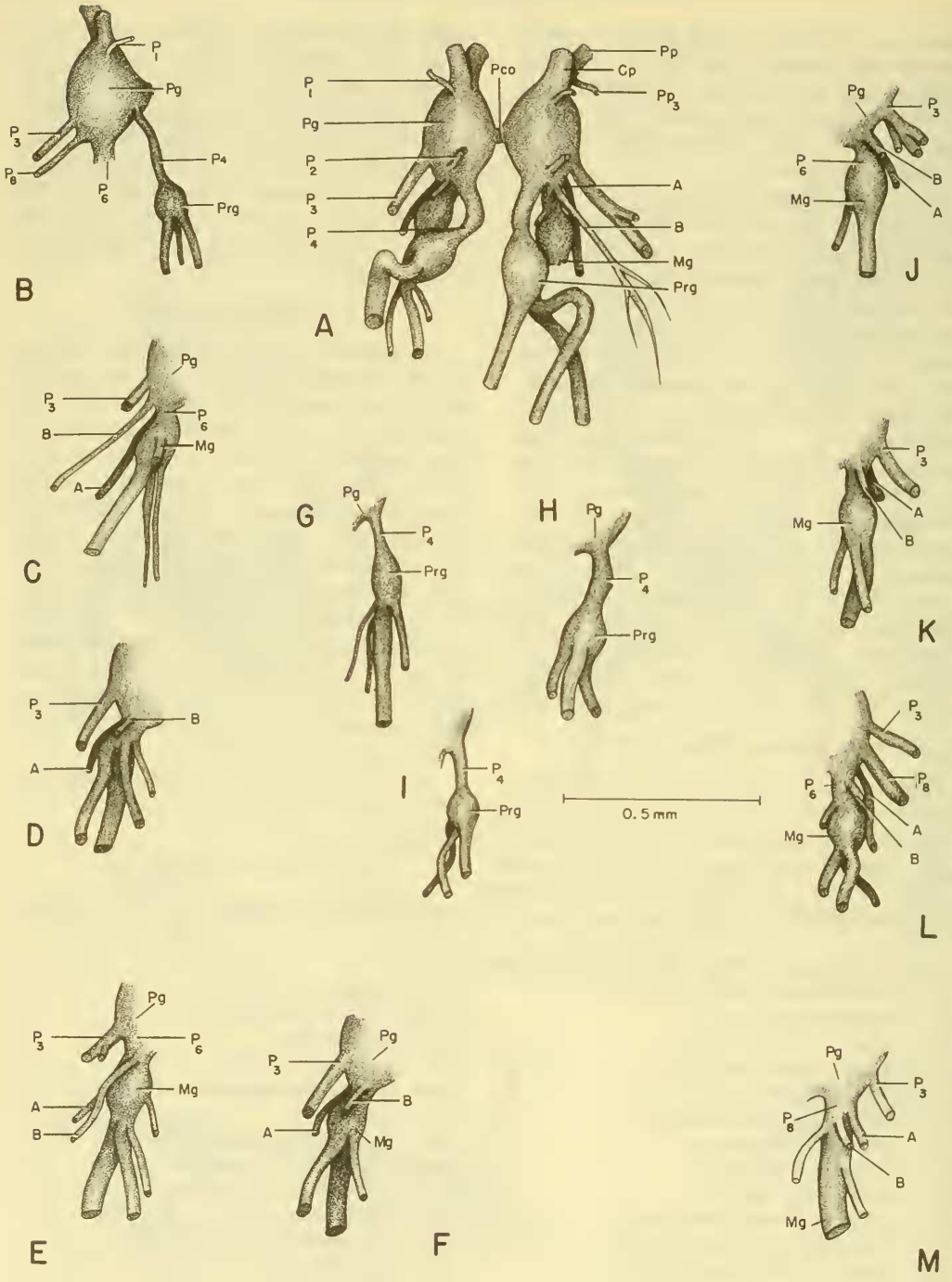
(1) Dorsal Aspect: The relationship of the cerebral ganglia to the buccal mass and esophagus is shown in lateral view in Fig. 11A. The dorsal aspect of the cerebral ganglia is shown in Fig. 12A. When the dorsal mid-line of the rostrum is opened, only the following nerves are observed: 1) optic (Opt), 2) tentacular (Tn), 3) supralabial (Sl), and 4) sometimes median labial 1 (Ml_1).

Dimensions of cerebral ganglion, cerebral commissure (Cc), and combined width of cerebral ganglia plus commissure are given in Table 4 in comparison with *Oncomelania hupensis formosana* and *Pomatiopsis lapidara*.

The only detectable differences be-

FIG. 12. Nervous system of *Oncomelania hupensis chiui*.
A. Dorsal aspect of central nervous system.
B-D. Variations in buccal ganglion complex.
E. Left pleural and subesophageal ganglia underlying the pleurosupraesophageal connective in Fig. A.
F-I. Variations in visceral ganglion complex.

b_1	dorsal buccal nerve	Mn_3	mantle nerve 3
b_2	esophageal nerve	Omn	osphradiomantle nerve
b_3	central buccal nerve	Opt	optic nerve
b_5	odontophoral nerve	Pn	pericardial nerve
Bg	buccal ganglion	Psc	pleuro-supraesophageal connective
Cb	cerebro-buccal connective	Rn	renal nerve
Cc	cerebral commissure	Rp	right pleural ganglion
Cg	cerebral ganglion	Sbv	subvisceral connective
Cl	columellar muscle	Sg	subesophageal ganglion
E_1	external mantle cavity nerve 1	Sl	supralabial nerve
E_2	external mantle cavity nerve 2	Sug	supraesophageal ganglion
E_3	external mantle cavity nerve 3	Sul	sublabial nerve
Gn	gonadal nerve	Suv	supravisceral connective
Lp	left pleural ganglion	Tn	tentacular nerve
Mcn	midcolumellar nerve	Tn_1	branch, tentacular nerve
Ml_1	median labial nerve 1	Var	variant nerves
Ml_2	median labial nerve 2	Vg	visceral ganglion
Mn_1	mantle nerve 1	Wall	left cephalic wall


P1
Pg
P3
P4
P8
P6
Prg
B
Pco
Pp
Cp
Pp3
P2
A
Mg
J
K
C
G
H
D
I
0.5 mm
L
E
F
M

tween the 2 subspecies of *Oncomelania hupensis* in dorsal view were: 1) generally, a "bulb" was lacking at the base of the tentacular nerve in *O. h. chiui;* it was present, though weak, in a few specimens only. Such a bulb is strongly developed in *O. h. formosana.* 2) The main branch of the tentacular nerve (Tn_1; Fig. 12A) arises at about midpoint on the tentacular nerve in *O. h. chiui* as it does in *Pomatiopsis lapidaria;* it arises from the base of the tentacular nerve in *O. h. formosana.*

(2) Lateral Aspect: The lateral complex involves the cerebral ganglion, pleuro-pedal and cerebro-pedal connectives. The right complex is shown in Figs. 11A, B. The lateral nerves are somewhat variable in position and number.

Lateral nerve 1 (Pp_1) is frequently not present (Fig. 11A vs 11B). Lateral nerve 2 (Pp_2) may be enlarged and later split into 2 at the lateral rostral wall. Lateral nerve 2 is the penial nerve in males. The origin of Pp_2 in *Oncomelania hupensis chiui* differs from that in *O. h. formosana,* where that nerve arises pressed against the origin of lateral nerve 3 (Pp_3). In *O. h. chiui* Pp_2 arises more dorsally from the pleuro-pedal connective (Pp). Lateral nerve 3 (Pp_3) arises and terminates the same way in both subspecies. Lateral nerve 4 described for *Oncomelania hupensis formosana* was not found in *O. h. chiui.*

In the left cerebral complex (Fig. 11D) Pp_1 is rarely present and when it is, it is very weakly developed.

(3) Cerebral Ganglion: The right cerebral ganglion is shown from exterior view in Fig. 11A (Cg). In Fig. 11C the medial surface is shown, to portray the exact position from which the cerebral nerves arise. The positions are the same as in *Oncomelania hupensis formosana.* Occasionally the cerebro-buccal nerve (Cb) is ventral to the sublabial nerve (Sul).

B. Pedal complex

(1) Anterior Aspect: The anterior aspects of the pedal, pro- and metapodial ganglia (Pg, Prg, Mg) are shown in Fig. 13. The greatest variability in the nervous system is found in the nerves (position and number) arising from or associated with the pro- and metapodial ganglia.

Consistent with *Oncomelania hupensis formosana* are the following:

(a) The elongate, cylindrical propodial connective (P_4).

(b) Nerves A and B arising, as shown, from the base of the pedal ganglion (Pg) or the beginning of the metapodial connective (P_6).

FIG. 13. Nervous system: pedal ganglion complex.

A. Anterior aspect of paired pedal ganglia and associated complex of nerves.

B. Varient in propodial connective showing an extra large lateral nerve (P_8) of the pedal ganglion.

C-F. Variation in nerves arising from right metapodial ganglion.

G-I. Variation in nerves arising from propodial ganglion.

J-M. Variations in the left metapodial complex.

A nerve from metapodial connective
B nerve from metapodial connective
Cp cerebro-pedal connective
Mg metapodial ganglion
P_1 lateral retractor nerve
P_2 nerve to antero-ventral wall of the pedal haemocoel
P_3 major lateral nerve of the pedal ganglion
P_4 propodial connective
P_6 metapodial connective
P_8 minor lateral nerve of the pedal ganglion
Pco pedal commissure
Pg pedal ganglion
Pp pleuro-pedal connective
Pp_3 lateral nerve 3 from pleuro-pedal connective
Prg propodial ganglion

TABLE 4. Comparison between forms of *Oncomelania hupensis* and *Pomatiopsis* of the sizes (in mm) of neural structures[4]

Neural structure	Dimension	Taxa		
		O. h. chiui mm	*O. h. formosana* mm	*P. lapidaria* mm
1. Cerebral ganglion (dorsal)	L	0.278-0.060	0.287*	0.290-0.360
	W	0.157-0.012	0.150*	0.238*
2. Cerebral commissure	L	0.073-0.097	0.070 ± 0.03	0.140-0.190
	W	0.061-0.097	0.06†	0.050-0.060
3. Total width of 2 cerebral ganglia and commissure	W	0.48-0.52	0.60*	0.76*
4. Pedal ganglion (anterior)	L	0.27†	0.24†	0.31†
	W	0.20†	0.22†	0.24†
5. Statocyst	D	0.09†	0.11†	0.12†
6. Buccal ganglion	L	0.14†	0.13†	0.19†
7. Pleural ganglion				
Right	L	0.154 ± 0.030	0.163 ± 0.300	0.240*
	W	0.105 ± 0.015	0.119†	0.120*
Left	L	0.121†	0.138†	0.170*
	W	0.121†	0.138†	0.170*
8. Pleuro-supraesophageal connective	L	0.287 ± 0.093	0.168 ± 0.050	0.34 ± 0.050
	W	0.054 ± 0.020	0.031†	0.041†
9. Supraesophageal ganglion	L	0.137 ± 0.016	0.100*	0.240*
	W	0.113 ± 0.014	0.080*	0.120*
10. Subesophageal ganglion	L	0.121†	0.138†	0.170†
	W	0.121†	0.138†	0.170†
11. Osphradio-mantle nerve (from "9" to the wall)	L	0.135 ± 0.030	0.412*	0.143*
12. Osphradial ganglion	L	0.385†	0.448*	0.571*
	W	0.125†	0.106*	0.142*
13. Visceral ganglion	L	0.159-0.227	0.283*	0.266*
	W	0.113†	0.133*	0.090-0.116

L = length
W = width
D = diameter
± = gives total variation

* = measurement from 1 individual
† = variability not encountered in measuring 6-7 individuals

[4] Measurements were taken from 6-12 adult individuals unless otherwise indicated. The total range is given without a mean value when only 6-7 measurements were made.

(c) The thin band-like metapodial connective (P_6) and often band-like metapodial ganglion (Mg).
(d) The pronounced major lateral nerve of the pedal ganglion (P_3).
(e) The irregular occurrence of P_8, the minor lateral nerve of the pedal ganglion.
(f) The position and strength of P_2, the nerve to the anteroventral wall of the pedal haemocoel.
(g) P_1, the lateral retractor nerve.

Nerves arising from the pro- and metapodial ganglia are very variable. Variation in the right metapodial ganglion and nerves is shown in Fig. 13, C-F; in the left metapodial ganglion, Fig. 13, J-M. Variation in the right propodial ganglion is shown in Fig. 13B, G-I.

(2) Lateral Aspect: The lateral aspect of the pedal ganglion, shown in Fig. 11A, corresponds with that of *Oncomelania hupensis formosana.* The lengths of the pedal ganglion and diameter of the statocyst in the 3 snails are compared in Table 4.

C. Buccal complex

The buccal ganglia are paired and connected to the cerebral ganglia by the cerebro-buccal connective (Cb; Fig. 11A).

Variation in nerves arising from the ganglion (Bg) are shown in Fig. 12, B-D.

The nerves are those found in *Oncomelania hupensis formosana.* The central buccal nerve (b3) is quite variable in position as shown in Fig. 12, B-D. The length of the right buccal ganglion is given in Table 4.

D. Pleural complex

The pleural ganglia (Lp, Rp) are shown in Fig. 11; 12A, E. These ganglia and associated nerves have the same shape and position as those in *Oncomelania hupensis formosana.* Their sizes are compared in Table 4.

The pleuro-supraesophageal connective (Psc; Figs. 11A, B, C; 12A) arises from the right plueral ganglion (Rp) and is noticeably longer and thicker than that found in *Oncomelania hupensis formosana.*

The most prominent nerve of the left pleural ganglion (Lp) is the mantel nerve (Mn_1; Figs. 11D; 12A, E) which runs postero-laterally to the cephalic wall where the mantel fuses with the wall.

The pleural ganglia are slightly smaller than those of *Oncomelania hupensis formosana* (Table 4).

E. Parietal complex

The parietal complex includes the supra- and subesophageal ganglia and the osphradial ganglion (Og; Fig. 10).

The supraesophageal ganglion (Sug; Fig. 12A) is more pronounced in *Oncomelania hupensis chiui* than in *O. h. formosana* (Table 4). From this ganglion arise 2 stout nerves or connectives; 1) the osphradio-mantle nerve (Omn; Fig. 12A) and 2) the supravisceral connective (Suv) to the visceral ganglion (Vg). The osphradio-mantle nerve runs 0.135 mm to the wall of the "neck," enters the wall as shown in Fig. 12A, and bifurcates, sending a mantel nerve to form a dialyneury with mantle nerve 1 (Mn_1) from the left pleural ganglion. The total length from supraesophageal ganglion to osphradium is 0.436 mm.

The relationship of subesophageal ganglion and left pleural ganglion is shown in Figs. 11D and 12A, E. The ganglia are either partially fused (Fig. 12E) or barely separated (Fig. 12A). The latter condition is more common and is what is normally found in *Oncomelania hupensis formosana.* As in *O. h. formosana,* 3 nerves, comparable in size and position, arise from the subesophageal ganglion: 1) mantel nerve 3 (Mn_3); 2) subvisceral connective (Sbv); 3) mid-columellar nerve (Mcn). The pronounced loop in the subvisceral connective (Sbv; Fig. 12E) is due to extreme contraction of the buccal mass. As shown in Fig. 12A, with protraction of the buccal mass the loop is pulled out. Comparison in length and width with *Oncomelania hupensis formosana* and *Pomatiopsis lapidaria* is given in Table 4. The ganglion is shorter and stouter than that of *O. h. formosana.*

F. Visceral complex

The position of the single visceral ganglion is shown in Fig. 5A. From the ventral aspect the ganglion (Vg) and 3 nerves are all that can be readily observed, i.e.,: 1) the subvisceral connective (Sbv), 2) the supravisceral connective (Suv), and, posteriorly, 3) the gonadal nerve (Gn).

With removal of some connective tissue and moving the position of the body and visceral ganglion slightly

TABLE 5. Comparison of radular size between 2 subspecies of *Oncomelania hupensis*

Feature	*O. h. chiui* (fr. 15 radulae)			*O. h. formosana* (fr. 16 radulae)			Significant difference
	$\bar{X}$	S	Se	$\bar{X}$	S	Se	
Radula: length (mm)	0.889	0.054	0.014	0.976	0.098	0.024	+ (P 0.01)
width	0.102	0.013	0.004*	0.120	0.008	0.002	+ (P 0.01)
Total number rows of teeth	77	4	1.03	84	7.5	1.9	+ (P 0.01)
No. rows of teeth in formative stage	15	4	1.03	19	5.3	1.4	None

*N = 11
$\bar{X}$ = mean
S = standard deviation
Se = standard error of the mean
+ = significant difference at 0.01 (1% level)

(Davis, 1967), one sees (Figs. 8; 12, F-I) that 3 nerves arise from the posterior ganglion and innervate various areas, as described for *Oncomelania hupensis formosana*. In addition to the gonadal nerve (Gn) there also arise the renal nerve (Rn) and external mantle cavity nerve 3 (E_3).

The pericardial nerve (Pn) varies in the position from which it originates from the supravisceral connective (Suv). As shown in Fig. 12, F-I, it most commonly arises about 0.11 mm from the visceral ganglion. A rare deviant is shown in Fig. 8, where the pericardial nerve arises from the visceral ganglion next to the root of the supravisceral connective.

The relationship of the visceral ganglion and associated nerves in males is shown in Fig. 9B. In both sexes the gonadal nerve travels posteriorly along the ventral side of the gonoduct.

Variations in the visceral ganglion and associated nerves are shown in Figs. 8; 12, F-I. Occasionally one finds fine nerve fibers passing to the mantel epithelium from the visceral ganglion (Var; Fig. 12G, H).

Exterior mantle cavity nerve 1 (E_1; Fig. 12F, G, I) corresponds to the same nerve in *Oncomelania hupensis formosana* in position and size. However, *O. h. chiui* lacks the strong kink in the subvisceral connective just posterior to the origin of E_1, found in *O. h. formosana*.

Exterior mantle cavity nerve 2 is not present in many specimens of *Oncomelania hupensis chiui*, though it is most often present in *O. h. formosana*.

The sizes of the visceral ganglia in the 3 taxa under comparison are given in Table 4.

9. Digestive System (Figs. 7; 11A, 14)

Three aspects of the digestive system are discussed in this section: 1) buccal mass, 2) stomach structure, 3) radula. Only the last is dealt with in detail.

The buccal mass has already been discussed in terms of external musculature above. It was thoroughly treated by Davis (1967) for *Oncomelania hupensis formosana*. The only aspect to be mentioned here is its length from rostral tip to cerebral commissure when the "mass" is fully contracted, which is slightly but distinctly smaller than that of *O. h. formosana* (see Table 3).

The ventral side of the stomach is

TABLE 6. A general formula for the most common cusp arrangement in *Oncomelania hupensis chiui* (from 30 radulae)

Tooth	General formula
Central ($\frac{\text{ant. cusps}}{\text{basal cusps}}$)	$\frac{2\text{-}1\text{-}2}{(3)2\text{-}2(3)}$
Lateral	2-1-3(4)
Inner marginal	7(8)
Outer marginal	6(7)

shown in Fig. 7, cleared of all overlying structures such as kidney tissue, reproductive organs (posterior section of prostate or pallial oviduct) and epithelium (Fig. 5A). The style sac (Sts), attached to the anterior chamber of the stomach (Ast), is conspicuous. The intestine (In) leaves the style sac as shown (Fig. 7) and discussed by Davis (1967). However, in that paper, the external morphology of the stomach is not so clearly shown.

The esophagus (Es) enters the stomach at the point of juncture of the posterior chamber (Pst) and anterior chamber (Ast). The single opening to the digestive gland is beneath the point where the vas deferens (Vd_1) crosses the boundary between digestive gland (Di) and stomach.

When the snails feed upon fine soil the fecal pellets (Fe) are rather uniform, solid, smooth and elliptical as shown in Fig. 7A. They are 0.40 ± 0.03 mm long and 0.18 ± 0.01 mm wide.

Radula (Fig. 14): Twenty radulae of laboratory bred snails were studied as well as 10 from field collected snails.

The length and width of the radula, the total number of rows of teeth, and the number of rows of teeth in the formative stage are compared with the same measurements for *Oncomelania hupensis formosana* (Table 5). The radula of *O. h. chiui* is distinctly smaller than that of *O. h. formosana*, i.e., it is shorter, more narrow, and has fewer rows of teeth.

TABLE 7. The various types of cusp arrangement for the different teeth in 30 radulae of *Oncomelania hupensis chiui* and the percentage of radulae showing that arrangement at least once

Central $\frac{\text{anterior cusps}}{\text{basal cusps}}$	%	Lateral	%
$\frac{2\text{-}1\text{-}2}{2\text{-}2}$	80	2-1-4 one side, 2-1-3 other side	50
$\frac{2\text{-}1\text{-}2}{3\text{-}3}$	70		
$\frac{2\text{-}1\text{-}1}{2\text{-}2}$	10	2-1-3	25
$\frac{2\text{-}1\text{-}2}{1\text{-}1}$	5	2-1-4	25
$\frac{2\text{-}1\text{-}1}{3\text{-}3}$	5	2-1-5	10
No. cusps inner marginal	**%**	**No. cusps outer marginal**	**%**
8	80	6	100
7	50	6 + 1	40
7 + 1	40	5	30
8 + 1	30	5 + 1	30
9	30	7	30
		7 + 1	20

+ 1 indicates the most lateral minute and often indistinct cusp.

In Table 6, the formula for the cusp arrangement most frequently encountered (i.e., occurring on 95% or more of the teeth on a radula or on different radulae) is given. The different types of cusp arrangements encountered at least once for each tooth are shown in Table 7.

The radula differs from *Oncomelania hupensis formosana* in that 1) the vast majority of centrals have 2 cusps on each side of the central anterior cusp while in *O. h. formosana* 62% of the radulae had

TABLE 8. Dimensional comparison for structures of the teeth (in μ) from field and laboratory reared *Oncomelania hupensis chiui*

Tooth	Feature	Laboratory (6 radulae)				Field (3 radulae)				Level Sign. diff.
		No.	$\bar{X}$	S	Se	No.	$\bar{X}$	S	Se	(P)
Central	A	12	25.2	2.11	0.61	7	26.3	2.42	0.91	-
	B	21	11.9	1.13	0.26	9	14.6	2.18	0.73	+ (.01)
	C	21	12.6	0.95	0.21	9	15.6	1.02	0.34	+ (.01)
	D	21	10.2	0.21	0.05	9	12.7	3.77	1.26	-
Lateral	L	16	45.8	1.44	0.36	6	47.3	2.09	0.85	-
	W	20	17.5	1.26	0.28	9	18.5	0.29	0.10	+ (.01)
Inner Marginal	L	21	40.9	2.84	0.62	8	45.4	2.63	0.93	+ (.01)
	W	30	16.5	1.08	0.20	7	15.7	0.75	0.28	+ (<.05)
Outer Marginal	L	21	42.9	2.70	0.59	5	46.6	3.20	1.42	+ (.02)
	W	14	13.7	0.96	0.25	7	14.6	0.98	0.37	-

A = width of base (posterior edge) of central tooth
B = anterior width
C = distance between tips of 1st basal cusps
D = distance from anterior edge of the tooth to the tip of the basal cusps
L = Length
W = Width

$\bar{X}$ = mean
S = standard deviation
No. = number of teeth measured
Se = standard error of the mean
P = probability level
- = not significantly different
+ = significant difference

but one in at least 90% of the individual teeth. 2) Many inner marginals of *O. h. chiui* have 7 cusps (50% of the radulae have this count at least once) while 8 or more are common in *O. h. formosana*. 3) In *O. h. chiui* the outer marginal has 7, or 7+1, cusps in 50% of the radulae at least once, while only 4-6 are found in *O. h. formosana*. Referring to 7+1, the +1 indicates the most lateral cusp which is often very minute and not distinct when the tooth is observed from certain positions. It is most evident when the tooth is bent back to expose clearly all cusps in one plane. 4) A cusp arrangement of 2-1-4 in the lateral teeth is common in *O. h. chiui* (at least once in 75% of radulae) while rare in *O. h. formosana* (10%).

While there is distinct overlap in cusp number, there are marked differences in frequency of cusp number as shown above. Of interest is the fact that in 50% of the radulae the lateral tooth had 2-1-3 cusps on one side of the central while having 2-1-4 on the other side. This arrangement extended the whole length (80%) or more of the rows where the formula could be discerned) of the radula. In *O. h. formosana* this asymmetry occurred in 10% of the radulae.

In studying the radulae of field snails, 2 distinct differences between these and laboratory reared snails were found: 1) in the size of portions of the central tooth, 2) in the cusp formula of the central tooth.

The dimensions for the teeth of the laboratory and field snails are given in Table 8. There was a significant difference in the 2 measurements of the central tooth: 1) width of the anterior edge of the tooth, 2) distance between the tips of the basal cusps (Bc; central 1, Fig. 14), which was greater in the field snails. Also, the width of the lateral tooth, as well as the lengths of the inner

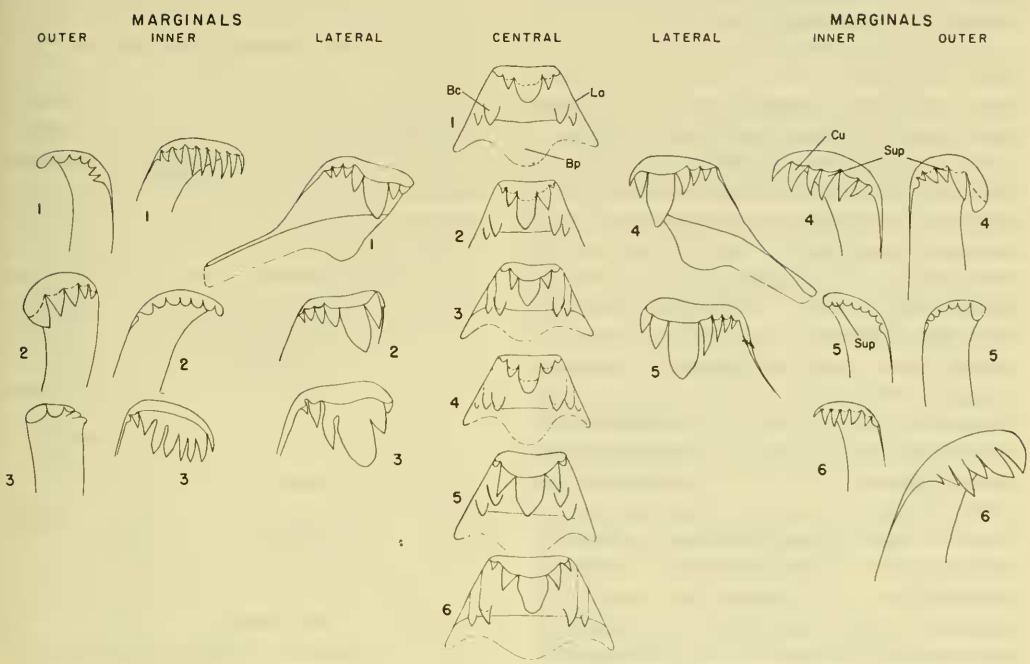


FIG. 14. A few teeth of each type found on the taenioglossate radula of *Oncomelania hupensis chiui* are given to demonstrate variation in size, shape and structure. Each cusp of a tooth is composed of a support (Sup) and dagger-like cutting edge (Cu). The supports are thickened; depending on the plane of focus, they may not be distinct (outer marginal 6) in which case the ends of the supports are represented by broken lines (e.g., outer marginals 2, 4, etc.). At another level of focus they are evident (e.g., inner marginal 4, laterals 1, 4, etc.).

The cutting edges of several cusps may grow out beyond the basal supports and fuse to form a bizzare enlarged cutting edge, e.g., inner marginal 3, lateral 3.

In preparing the radula for study, cutting edges may have been accidentally broken away from the supports, e.g., in outer marginals 1, 3, 5, etc.

Bc	basal cusp	Cu	cutting edge of the cusps
Bp	basal process attaching central to lingual membrane	La	lateral angle of the central tooth
		Sup	support for cutting edge

and outer marginals, in the field snails were significantly greater (P at the .01 level) whereas the width of the inner marginal of the field snails was significantly less than that of the laboratory snails ($P < .05$).

In 1 or 2 field snails a central cusp formula of 2-1-2/4-4 was found. The 4th basal cusp on each side was tiny but distinct.

Occasionally one of the marginals was larger than usual in the field snails (outer marginal 6, Fig. 14).

The teeth shown in Fig. 14 are presented to show variation. In some instances the cutting edge (Cu) of the more pronounced cusps of the lateral or inner marginals grew considerably beyond the basal supports (Sup) to form a wide, fused cutting edge with pronounced

jagged denticles (lateral 3, inner marginal 3 contrasting with the more normal lateral 1, inner marginal 1).

10. Conclusion

On the basis of this anatomical study and detailed comparison with *Oncomelania hupensis formosana*, it is evident that the so-called *"Tricula chiui"* is, indeed, a member of the *Oncomelania hupensis* complex. With a few minor exceptions, qualitative aspects of anatomy are the same. The exceptions are: 1) lack of nerve Pp_4 (lateral nerve 4 from the cerebro-pleural complex) present in *O. h. formosana* but not demonstrated in *O. h. chiui*. This nerve was not always demonstrated in *Pomatiopsis lapidaria* or *O. h. formosana* and when it was not found it was "suspected that it was incorporated within the pleuro-pedal. commissure" (Davis, 1967). 2) The origin of nerve Tn1 from the midtentacular nerve instead of the base of the nerve. 3) The number and arrangement of medio-lateral slips of the buccal protractor (M_5) as discussed in the section on the muscular system (p 32). Details of female and male anatomy are identical in the 2 snails. Differences in structure compared with other genera (mainly *Pomatiopsis*) are those discussed by Davis (1967).

Most differences from *Oncomelania hupensis formosana* are connected with the smaller size of *O. h. chiui*, i.e., the organs are generally smaller. All such differences are thus considered a correlate of 1 factor, smaller size.

Nevertheless there exist several differences important enough to assign the taxon to subspecific rank apart from *Oncomelania hupensis formosana*.

1. Although the length of gill in the 2 subspecies is the same, *O. h. chiui* has distinctly fewer gill lamellae (under 36 as against over 42).

2. The pleuro-supraesophageal connective of *O. h. chiui* is distinctly and significantly longer.

3. The supraesophageal ganglion is distinctly of greater size in *O. h. chiui*.

4. There are differences in the frequency with which different cusp numbers occur on the various teeth on the radula, that are thoroughly discussed in the section on the radula.

5. The shell has potential for forming only a very vague varix.

These 5 major anatomical differences together with the uniformly smaller size would be taken by many to justify full specific status. The point will be further discussed below when hybridization, electrophoretic and serological data are considered.

TABLE 9. Cultures involving the mating of *Oncomelania hupensis chiui* with *O. h. formosana* and *O. h. quadrasi* and their productivity

Subspecies of *Oncomelania hupensis*			Duration[5] (months)	Young/ female/ day
chiui		*formosana*		
5 ♀	x	5 ♂ (Yueh Mei)	9	0.12
5 ♀	x	5 ♂ (I-lan area)	9	0.05
5 ♂	x	5 ♀ (Pu Yen)	7	0.22
5 ♂	x	5 ♀ (Pu Yen)	7	0.26
5 ♂	x	5 ♀ (Yueh Mei)	7	0.29
5 ♂	x	5 ♀ (Yueh Mei)	7	0.12
chiui		*quadrasi*	5	
2 ♀	x	5 ♂		0.00

[5] The cultures were started in January, March and May, and were all terminated in October, 1965.

HYBRIDIZATION STUDIES

1. Materials and Methods

Five males or 5 (2) females of *Oncomelania hupensis chiui*, resp. of *O. h. formosana* of various strains and also 5 male *O. h. quadrasi* were placed in cross cul-

ture as shown in Table 9. Males were uniformly 3-4 months old. Females were reared singly in Petri dishes as described by van der Schalie & Davis (1965) from the 2.5 whorl stage. Females were maintained in isolation until an age of 2.5 months and were then placed in culture.

The breeding vivarium was the medium clay pot, kept at room level light, as described by Davis (1967) and van der Schalie & Davis (1968). Cultures were checked monthly for young. Each month the sex of all snails was checked in all cultures to ascertain whether a male of the wrong subspecies had been erroneously included. All young were removed each month.

2. Results

As shown in Table 9, all cultures produced young, except the cross involving *Oncomelania hupensis quadrasi*. The rate of production varied from 0.05-0.29 young per female per day over the entire period of 7-9 months, when the crossmatings involved *O. h. formosana*.

3. Discussion

This series of crosses provides no more than initial data on the potential of *Oncomelania hupensis chiui* to form hybrids with the other subspecies of *Oncomelania hupensis*. The negative results obtained with *O. h. quadrasi* are not reliable in view of the low number of females and the single culture involved.

The rate of reproduction was at a level corresponding to that for *O. h. formosana* reared under identical conditions. The latter produced 0.17-0.33 y/f/day in their first year in culture (van der Schalie & Davis, 1968).

These initial results substantiate that *Oncomelania hupensis chiui* is, indeed, closely related to *O. h. formosana*. Further crossing studies should be done to assess relationships to the other subspecies of *Oncomelania hupensis*.

ELECTROPHORETIC ANALYSIS

1. Introduction

The value of electrophoresis is covered in terms of data and literature survey in the reference volume Taxonomic Biochemistry and Serology (1964). Cheng (1964) reviewed some previous work pertaining to electrophoreses and molluscan systematics; Davis & Lindsay (1967) provided other references. Additional papers are those of Targett (1963), Wright & Ross (1966) and Wright, File & Ross, (1966).

The present study involves comparing the electrophoretic "fingerprint" patterns of *Oncomelania hupensis chiui* with those of 3 field collected populations of *O. h. formosana:* 2 from I-lan county (church and airport populations) and 1 from the Pu Yen village area, Changhua county (Fig. 1). In addition, laboratory reared F_1 and F_2 generations of I-lan parental stock (church population) were studied.

The advantages and disadvantages of using polyacrylamide electrophoresis for this type of study are discussed by Davis & Lindsay (1967). As stated by them this method has the advantages of being sensitive enough to demonstrate variation between populations of a species when such variation occurs. High resolution of numerous protein fractions (15-26) is obtained in a sample with as little as 200-300 micrograms of protein.

2. Materials and Methods

The source of proteins was foot muscle extract. Foot tissue from 20-50 snails was pooled for homogenization as discussed by Davis & Lindsay (1964, 1967) and Davis (1967). Justification for pooling tissue was discussed by them.

Polyacrylamide (=disc) electrophoresis was employed. The standard 7.5% acrylamide gel and tris-glycine buffer (pH 8.2-8.4) were routinely used as discussed by Davis & Lindsay (1967). From 3-10 experiments were made for each population. Each experiment comprized 5-10 gel tubes. Human blood serum was used in controls (see below) for determining whether the gels of each experiment were optimal, good or poor. Gels were stained in amidoschwartz for 2 hours and destained electrically in 7.5% acetic acid. Stained gels (uniformly 34-35 mm long) were analyzed for densi-

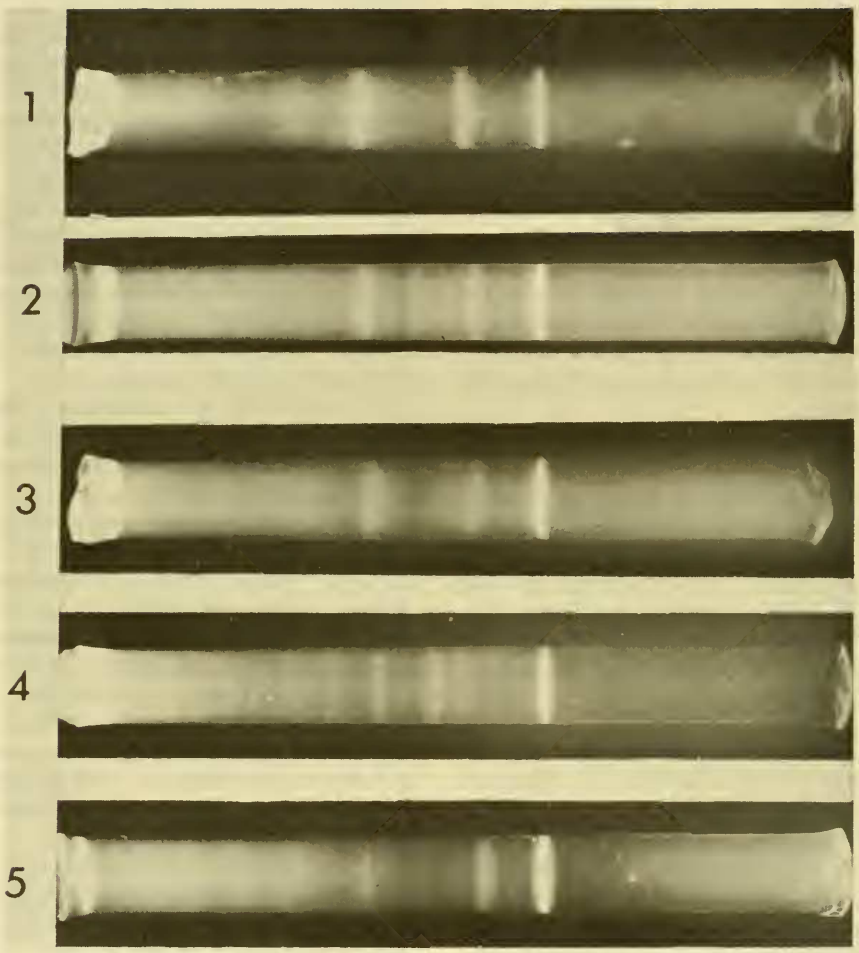


FIG. 15. Disc-gel tubes with characteristic electrophoretic separation patterns for foot tissue proteins from the 5 *Oncomelania* populations studies. The stained gels (34–35 mm long) were placed directly under an enlarger and the distribution pattern was printed, with the gel column serving as a negative.

1. *Oncomelania hupensis chiui.*
2. *O. h. formosana,* I-lan church population.
3. *O. h. formosana,* I-lan airport population.
4. *O. h. formosana,* Pu Yen population.
5. *O. h. formosana,* I-lan laboratory population.

tometric patterns by means of a Canalco Model E microdensitometer.

Data are discussed in terms of optical densitometric pattern and of Rf values (ratio of distance from the origin to a given fraction to distance from origin to the front). The former is the result of component position and density while the latter indicate only component position. Methods used for determining Rf values

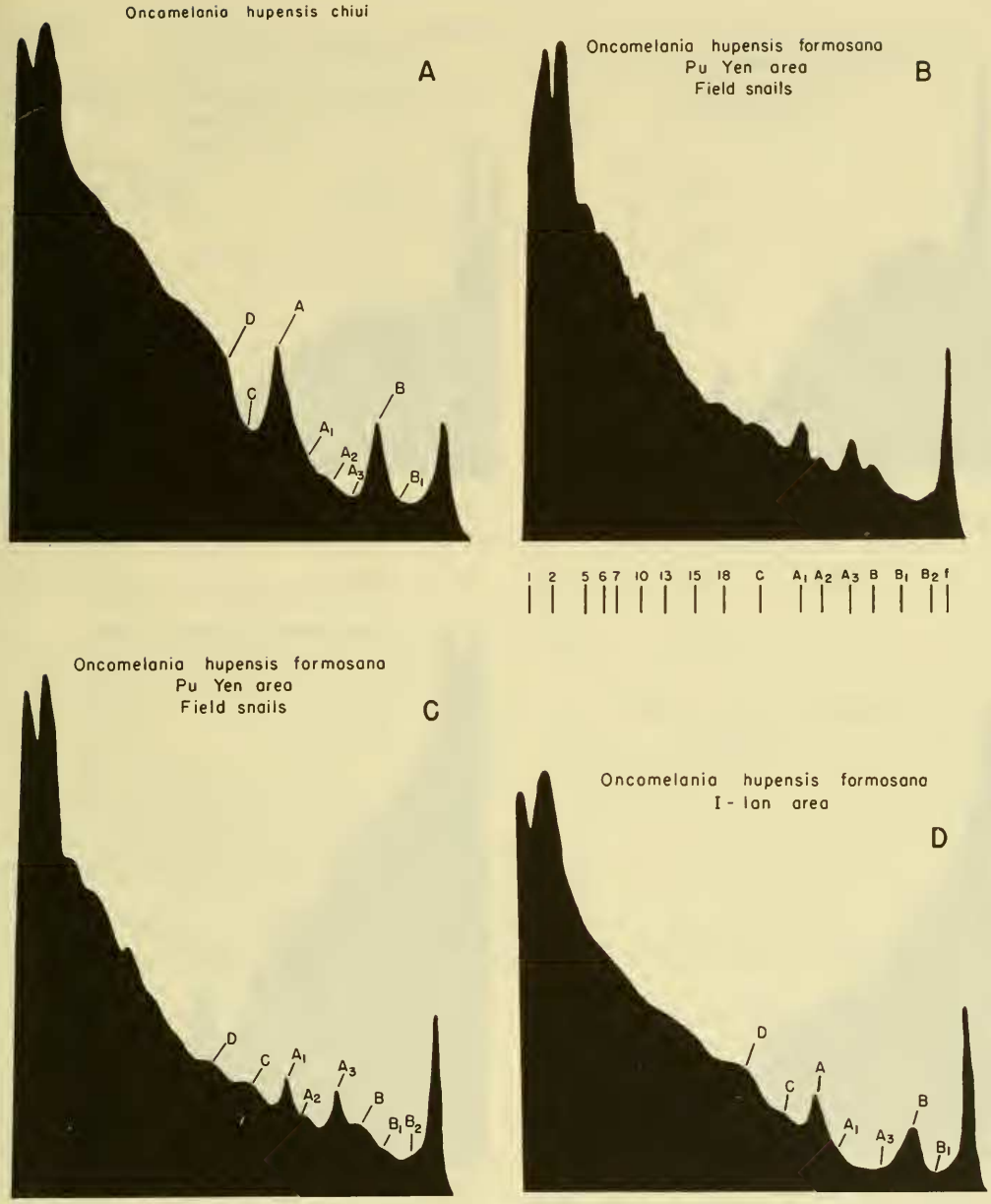


FIG. 16. Densitometric tracings of the electrophoretic protein distribution patterns of 3 *Oncomelania* populations. Letters marked in Figs. A, C, D (and right half of B) are used in the text to discuss profile patterns as well as component position in the frontal, most characteristic part of the profile (gel area 2). In Fig. B the individual components in the initial part of the profile (gel area 1) are numbered. The profile given in C is of a different run than the one shown in B; it serves to demonstrate reproducibility of profiles from different experiments and aids in comparing the different types of densitometric pattern found in A and D. Fig. D is a generalized pattern for the I-lan field snails.

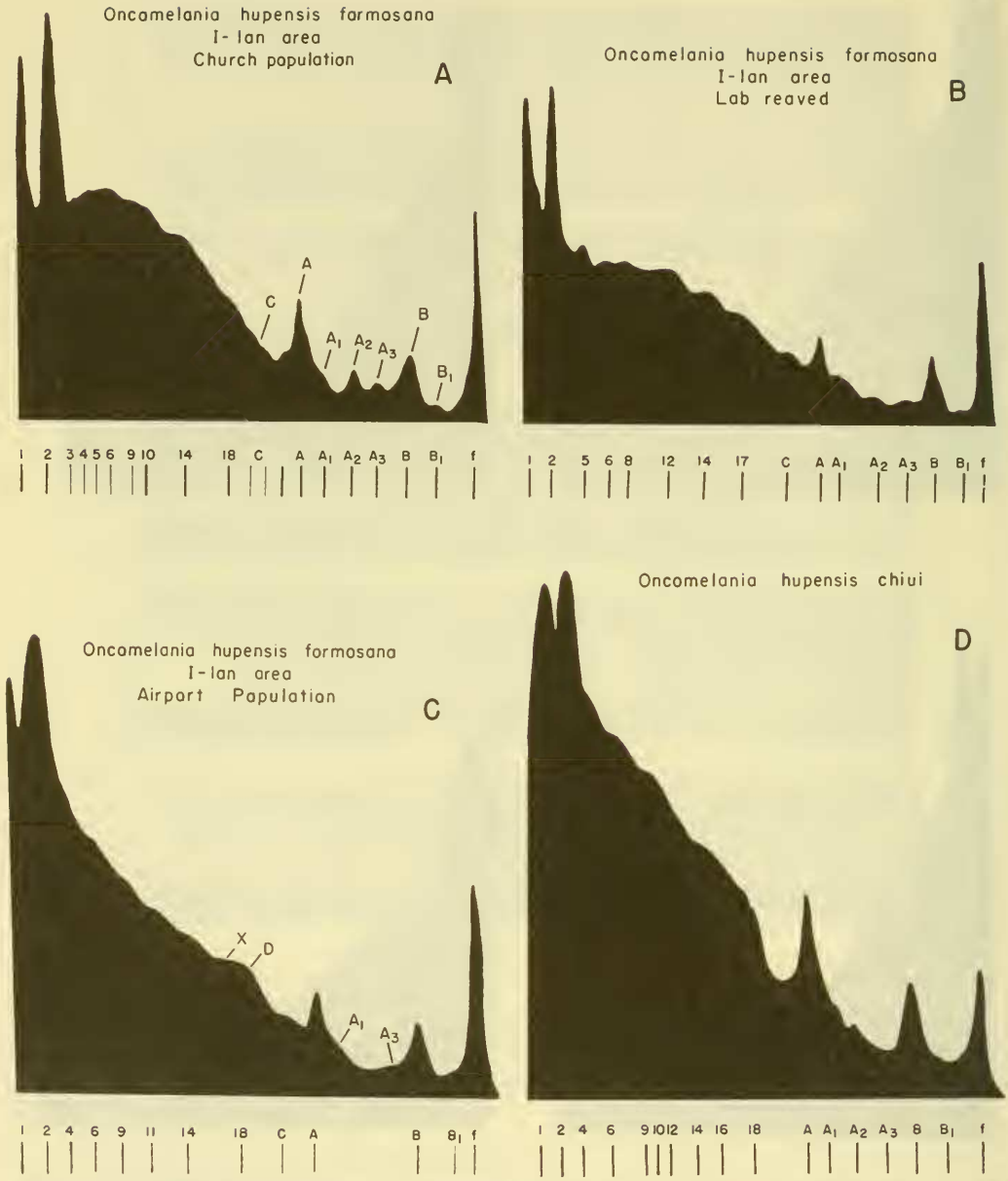


FIG. 17. Densitometric tracings of the electrophoretic patterns of 4 *Oncomelania* populations. The position of each protein component is marked by numbers in gel area 1, and by letters in gel area 2. The same symbol, in the different populations, indicates the same Rf value for the components thus represented.

TABLE 10. Average Rf values for components in gel area 1[6], for the 5 populations of *Oncomelania hupensis* studied

Component No. in gel area 1	*O. h. chiui*	*O. h. formosana*			
		I-lan			Pu Yen
		Church	Airport	Lab	
1	.015	.014	.014	.014	.014
2	.064	.066	.066	.065	.071
3	*	*	*	*	*
4	.117	.115	.115	*	-
5	*	.138	*	.138	.133
6	.174	.188	.179	.176	.183
7	-	-	-	-	.211
8	-	-	-	.223	-
9	.247	.244	.230	-	-
10	.276	.271	-	-	.266
11	-	-	-	-	-
12	.303	.290	.288	.291	-
13	-	-	-	-	.326
14	.373	.377	.368	.377	-
15	-	-	-	-	.392
16	.247	-	-	-	-
17	-	-	-	.456	-
18	.477	.478	.472	-	.465
No. of component differences compared to *O. h. chiui:*		1	2	5	7

* = fractions resolved only under optimal gel conditions.

[6] Area of dense, slowly migrating, large molecular weight proteins (see Figs. 16, 17). The average valves were derived from 8-10 tubes from 3-10 different experiments.

are discussed by Davis & Lindsay (1967).

Rf values were determined for each fraction in all tubes and are presented as averages. As separations of proteins from homologous preparations yield stable patterns, the standard deviation for an average Rf value rarely exceeded the error arising when 2 persons measured the same component, i.e., 3%, or an Rf value of 0.014. Total variation in a long series of measurements from a number of homologous runs rarely exceeded 0.045 and more commonly was 0.020 or less. It was determined that, in general, when average Rf values differed by 0.018, they were very significantly different statistically. When Rf values are 0.017 apart, it is necessary to determine by calculation whether or not they are significantly different.

3. Results

The typical electrophoretic patterns for each of the 5 populations investigated, i.e., the stained gel columns themselves, are shown in Fig. 15. For ease in comparing the densitometric profiles (Figs. 16, 17) drawn from typical electrophoretic separations, the linear sequence of components was divided into 2 areas. Area 1 extends from the origin (fraction 1) to point D (resp. fraction 18). In this stretch of gel are numerous closely

TABLE 11. Average Rf values for components in gel area 2[7] for the 5 populations of *Oncomelania hupensis* studied

Component in gel area 2	*O. h. chiui*	*O. h. formosana*			
		I-lan			Pu Yen
		Church	Airport	Lab	
C	.544	.550	.546	.557	.555
A	.601	.614	.632*	.624*	-
A_1	.652	.657	-	.674*	.645
A_2	.721	.729	-	.751**	.705
A_3	.780	.786	-	.823**	.762*
B	.831	.857*	.859*	.881**	.821
B_1	.896	.914	.923*	.940**	.888
B_2	-	-	-	-	.969*
Front	1.00	1.00	1.00	1.00	1.00
No. of component differences compared to *O. h. chiui:*		1	6	6	3

* = Rf significantly different from *O. h. chiui*

** = Rf significantly different from both *O. h. chiui* and the church population

[7] Area of more motile proteins with lower molecular weight (see Figs. 16, 17). The average values were derived from 8-10 tubes from 3-10 different experiments.

packed protein fractions of relatively high molecular weight; they are dense and slowly moving components. This region is characterized by a tendency for a dark gel background due to diffuse protein complexes.

Area 2 starts at C (Figs. 16, 17) and continues to the front (f). It contains rapidly migrating proteins which are usually not extremely dense. In this second area fractions tend to be more widely spaced and distinct. Area 2 is characterized by relative stability in fraction density and resolution under gel conditions causing distortion, diffusion of components, or difficulty in resolving components in area 1.

Variance in sample concentration, gel conditions, and length of electrophoretic separation have an effect on the number and resolution of components in area 1. In human blood controls optimal gel conditions resulted in resolving over 22 fractions and resolution of post albumen fractions (bands between transferrin* and albumen) was excellent. Less than optimal conditions resulted in resolving only 19-20 fractions while post albumens were fuzzy or 1-2 were absent. When 19-20 fractions of human serum proteins were resolved and all post albumens were observed, gel conditions are considered good. Most results presented here were obtained when gel conditions were good or optimal. With optimal conditions several more bands were resolved in area 1 (if run lengths were the same). For instance, component X (Fig. 17C) could, at times, be resolved from the relatively wide, diffuse fraction resulting in densitometric peak D.

The average Rf values for components in the initial, dense region (gel area 1)

* Transferrin is the iron binding protein in serum that is very prominent in polyacrylamide electrophoresis when serum components are being separated. The component was shown by Ornstein (1962) in relationship with other human serum components.

for the populations studied are presented in Table 10. The following differences in the congruency of components were noted between *O. h. chiui* and the other populations: 1 difference from the I-lan church population, 2 and 5 differences from I-lan airport and laboratory populations respectively; there were 7 differences from the Pu Yen field population.

Certain bands (marked with an asterisk in Table 10) are resolved only when gel conditions and protein concentrations are optimal. In all populations proteins between Rf values of 0.392 and 0.477 may appear blurred, with denser areas representing band centers. These fractions are only clearly resolved under optimal gel conditions and even then there is a stained, blurred area between the components.

In the frontal gel area 2 there are 2 different types of densitometric pattern found in the 5 populations. Type I includes *O. h. chiui* and all populations of I-lan *O. h. formosana* (Figs. 16A, D; 17A-D). Type II corresponds to the Pu Yen population of *O. h. formosana* (Figs. 16B, C).

In the former pattern (Type I) the 2 fractions at positions A and B are widely separated, dense components. Between A and B are 3 minor fractions: A_1 - A_3. In the latter pattern (Type II), 3 distinct, dense components are observed, but they correspond to positions A_1, A_3 and B. The density of the fraction at B is regularly quite less than that of the fractions A_1 and A_3 (Fig. 16B, C).

The fraction at position C in Type I is not dense, and in *O. h. chiui* it is frequently not resolved. The result in densitometric pattern due to this faint fraction is a marked dip between position A and D (D represents the end of gel area 1) (Fig. 16A). As shown in Table 12, fraction C was not resolved in more than 75% of the tubes of experiments yielding a Type I densitometric pattern. In the Type II pattern (Pu Yen population) the fraction at position C was present in 100% of the tubes of all experiments and was characteristically quite dense (Fig. 16B, C), often appearing as 2 dense, slightly separated bands.

TABLE 12. The percentage of tubes of all experiments in which component C was resolved for the 5 populations of *Oncomelania hupensis* studied

Snail populations studied	%
O. h. chiui	57
O. h. formosana	
I-lan populations:	
Church	75
Airport	75
Laboratory	69
Pu Yen population	100

Considering the Type I pattern, fractions at positions A_1 - A_3 were distinct for *O. h. chiui* but at a very low density. Component A_1 had a tendency not to resolve into a distinct band and often appeared as a diffusely stained area closely associated with A. A_2 was never resolved in the airport population of *O. h. formosana* (Fig. 17C) while A_1 and A_3 were either faint diffuse bands or unresolved. A_1, A_2 and A_3 were all distinct and of moderate density in the church population (Fig. 17A).

Component positions A, B, C, etc. were used in discussing reference points in the 2nd area of the densitometric tracings or patterns. The actual Rf values for the fractions pertaining to these patterns are given in Table 11. The average Rf values of the various populations of *O. h. formosana* are compared with those of *O. h. chiui* and the number of corresponding fractions is listed. It is seen that Rf values corresponding to the same peak in the densitometric tracings can significantly differ. At position B, for example, *O. h. chiui* significantly differs from all I-lan snails, while the laboratory population, descended from the church I-lan snails, significantly differed from both the field snails and *O. h. chiui*.

On the basis of Rf values alone, in the 2nd gel area, *Oncomelania hupensis chiui* most closely corresponds to the I-lan church population (1 difference) and least of all, to the laboratory and airport populations (6 differences). The low correspondence of fraction position between populations of I-lan snails will be discussed later. The Pu Yen snails are separated from all the other populations on the basis of a different densitometric profile, i.e., different fraction position and density.

Oncomelania hupensis chiui has distinct components at Rf 0.117, 0.174 and 0.247. Rarely is a faint band resolved at 0.138; in this respect it differs from the church and laboratory I-lan populations of *O. h. formosana* where Rf 0.138 is very prominent.

4. Conclusion and Discussion

The results indicate that electrophoretically, *Oncomelania hupensis chiui* is nearly identical with the church population of I-lan *O. h. formosana*.

Basically, the electrophoretic patterns of all populations studied are quite similar. As previously mentioned (Davis, 1967), all the subspecies of *Oncomelania* are characterized by at least 1 dense, distinct fraction at an Rf beyond 0.750. In the American *Pomatiopsis lapidaria*, a hydrobiid snail most closely related to *Oncomelania*, there are no dense fractions beyond 0.750, and, although there are other distinctive features, this difference is the most outstanding.

As already stated, the Pu Yen population of *Oncomelania hupensis formosana* differs from the others, in gel area 2, in pattern, i.e., both in density of fraction and Rf. These differences, coupled with the many (7) differences in Rf in area 1, sets the Pu Yen population aside from the others.

Concerning the I-lan field populations of *O. h. formosana*, the airport population appears different because of the lack of distinct A_1 - A_3 fractions in gel area 2. As mentioned above, A_1 and A_3 are present as diffuse bands, while A_2 was not resolved. In the laboratory population the whole pattern of C to B_1 has shifted towards the front, relative to the church population, so that the Rf values appear different. The pattern itself, however, remained the same, indicating homology of fractions despite a global shift. In the I-lan populations of *O. h. formosana* (laboratory and field) and in *O. h. chiui*, there is a tendency for increased resolution of A_1 - A_3 as follows: from poor in the airport population, and slightly more pronounced in the laboratory population and *O. h. chiui* to very pronounced in the church population.

The laboratory population appears different from the field populations because of the above mentioned global shift of fractions. It should be further tested to what extent laboratory bred populations, always descended from a narrower selection, differ from field snails. One would conjecture that changes in electrophoretic patterns would range from none, or very slight, to a degree indicating a population difference as marked as that separating the Pu Yen field snails from the I-lan snails. It is doubted, however, that a difference tantamount to a species difference would occur.

The electrophoretic differences observed between these populations are reproducible and characteristic. A discrete difference indicates a taxon difference. Assigning such differences to categories of species, subspecies, or population variants, depends on 2 groups of facts: 1) On how electrophoretic data fit in with those involving other characters; 2) On the magnitude of electrophoretic difference found between taxa which are accepted as distinct on many criteria, yet clearly belong within the same genus.

The assignment of these populations to categories is reserved for the final discussion, where electrophoretic considerations will be a factor among others in assessment.

IMMUNOLOGICAL STUDIES

1. Introduction

Immunological studies were undertaken to determine the relationship between *Oncomelania hupensis formosana* and *O. h. chiui* in terms of homogeneity or heterogeneity of antigens. The comparison also involved 3 other subspecies of *O. hupensis*.

Relatively few papers deal with immunological studies on mollusca that involve systematic relationships or taxonomy. Pertinent references are those of Morrill, Norris & Smith (1964), Michelson (1966a, b) and Wright & Klein (1966).

2. Materials and Methods

a. Antigen preparation and production of antisera

The source of antigens was foot muscle extract of *Oncomelania hupensis formosana* from Pu Yen, prepared exactly the same way as if it were to be used for electrophoresis (Davis & Lindsay, 1967). Only freshly prepared extracts were used in all experiments and to produce antisera. The extract in the immunodiffusion experiments was prepared as follows: 0.30 gm of blotted wet weight of tissue was homogenized in 2.0 ml Carriker's Saline as described by Davis & Lindsay (1967), the homogenate was centrifuged at 250 xg for 5 minutes and the supernatant (= extract) decanted. All operations were carried out at 2-5° C. The protein content of the extract was determined using the Biuret reaction (fide Kabat & Mayer, 1961) and a standard curve was established using purified crystalline bovine albumen (clinical pathology standard). Readings from the Biuret reaction were made with a Shimadzu QR-50 spectrophotometer. Snail foot extract, as prepared above, yielded 6.0-7.0 mg/ml of protein.

White rabbits (5-6 lbs; virgin female) were used to produce antisera. Foot tissue extract was prepared at the concentrations indicated in Table 13, column 2. The stated weight of tissue was homogenized per 1 ml saline and treated as described above. The extract thus obtained was injected into the rabbit intravenously via a lateral ear vein, in 2 injection series, 2 months apart (see Table 13 for schedule). A total of 7-8 mg of protein was injected in each series. Rabbits were bled from the ear 5 and 10 days after the last injection in the second series. It had been previously established that the strongest and best defined antigen-antibody precipitin systems resulted from serum obtained 5 days after the last injection, and that these were somewhat weaker with 10th day serum.

Antiserum was pressed through a sterile millipore filter (0.45μ) into presterilized tubes. Merthiolate (aqueous 1:1000) was added in the proportion of 1:10 as a preservative and the serum was stored at 3° C for the duration of the experiments.

Although 4 or 5 rabbits were used to produce antiserum, the 5th, resp. 10th day antiserum from only 1 of these was used in all experiments here reported, because tests had revealed their greater specificity and higher quality, as evidenced by the number and strength of precipitin systems.

b. Diffusion Techniques

Two tests were utilized in this study. (1) Micro-Ouchterlony double diffusion procedures were carried out; these made it possible to test through in-gel specific absorption of antiserum by antigens (and consequent precipitation), the homogeneity or heterogeneity of relevant antigens in the foot muscle extracts of a population, with regard to the corresponding antigens from Pu Yen snails. (2) Immunoelectrophoresis was used to demonstrate the position of separated relevant antigens in the acrylamide gel columns, so as to permit a comparison of the protein bands making up a taxon-specific densitometric profile (discussed in the electrophoretic section) and the

TABLE 13. Schedule for intravenous injections of snail[8] foot muscle extract into rabbits for antibody production

Time schedule (Days)	Foot tissue homogenized mg blotted wet weight per 1.0 ml saline	mg protein injected	
		$\bar{X}$	Se
1	5.55	0.60	0.143
3	13.69	0.78	0.145
5	27.75	1.06	0.066
7	55.50	2.46	0.156
9	83.25	2.75	0.000
2 month interval		7.65 total	
1	5.55	0.60	0.143
3	13.69	0.78	0.145
5	27.75	1.06	0.066
7	55.50	2.46	0.156
9	83.25	2.75	0.000
14 (=5th day) bled from 19 (= 10th day) the ear		7.65 total grand total 15.30	

$\bar{X}$ = mean value
Se = standard error of the mean

[8] *Oncomelania hupensis formosana*, Pu Yen population

position of antigens which were involved in the double diffusion tests (it will be remembered that snail foot extract was prepared in the same way for both electrophoresis and immunological specific absorption tests).

Agar, used in both types of tests, was prepared as follows: Non-nutrient agar (Difco Company, Detroit, Michigan, U. S. A.) was made up to 2% in distilled water, cut into blocks, and rinsed in cold water for 12 hours. The agar was then stored in distilled water in the refrigerator for several days, changing the distilled water daily, for removal of soluble impurities. In preparation for the tests the 2% agar was dissolved in 0.90% saline to form a 0.45% final saline concentration, merthiolate was added as a preservative to a final concentration of 1:10,000 and the final concentration of the agar was 1%.

In all tests, extracts and antisera were used undiluted. When the tests were initiated by permitting double diffusion (i.e., of antigens and antibodies) to commence, the experiment proceeded in a moist environment for 3 days at 21° ± 1°C (3-5 days for immunoelectrophoresis) to allow for proper diffusion and precipitation. Resulting opaque precipitin patterns were recorded by placing unstained gels under a photographic enlarger and using them as negatives. The arc patterns were thus printed out on photographic paper and are reproduced in Figures 18-25. Tracings were also made of the precipitin arcs so as to safeguard against loss of detail in final reproduction, especially as several systems were light and closely associated.

Control serum did not react with saline or extract.

1) Specific absorption tests (based on double diffusion principles and using micro-Ouchterlony techniques). Tests were conducted in agar contained in

rings (inside diameter 22 mm), cemented to microscope slides (75 x 25 mm) with a paraffin-wax mixture. Agar (1%) at 60°C was poured into the rings (2.5 ml/ring) and allowed to solidify. Four wells arranged in a diamond pattern were cut in the agar (Figs. 19-25) using a template designed by Dr. George Nace, University of Michigan, Ann Arbor. The centers of the wells on the long axis were 16 mm apart, those on the short axis 12 mm apart. Each well had a diameter of 4 mm and a capacity of 82-85 μl.

The immunodiffusion tests were conducted as explained in the following example, where 2 populations of *Oncomelania hupensis formosana*, one from Pu Yen and one from Yueh Mei, and anti-Pu Yen serum were used (Fig. 19B). Foot tissue extract of snails from Yueh Mei (heterologous extract, since the homologous systems involve extract of Pu Yen snails and anti-Pu Yen serum) was placed in well labeled Abs-YM. At that time no other wells were filled. The extract was allowed to diffuse through the gel for 12 hours at which time components had migrated to a distance of 1.5-2.0 mm from the well. At 12 hours the excess extract was removed from the well, thus slowing considerably the rate of diffusion of the extract. Then, fresh heterologous extract from Yueh Mei snails was placed into well YM and fresh homologous Pu Yen snail extract was placed into well PY while anti-Pu Yen serum was placed into the well marked Abs-YM and into the unmarked well.

The antiserum in the Abs-YM well then diffused through the barrier of previously diffused heterologous Yueh Mei antigens around the periphery of the well with ensuing absorption of relevant antibodies. The absence of precipitin bands between wells Abs-YM and YM showed that absorption was complete, i.e., that all relevant antigens of the Yueh Mei extract that could react with anti-Pu Yen serum had precipitated out around well Abs-YM. The presence of bands between the Abs-YM and PY wells (compare Figs. 23-25) would indicate antigens unique in, or partially particular to, the Pu Yen snails. The absence of such bands in Fig. 19B indicates that the antigens of the 2 populations here investigated were homologous. In contrast, precipitin arcs did result from the interaction of the non-absorbed antiserum diffusing from the unmarked well (bottom), which had not previously contained antigen, and the antigens diffusing from the (lateral) wells marked YM and PY. The number of arcs which formed around the unmarked well indicate the number of antigens harboured in common by the 2 populations and reacting with anti-Pu Yen serum.

2) Immunoelectrophoresis. Unstained polyacrylamide gels (i.e., gel columns comprizing the "native" components electrophoretically separated in the "lower" gel (to the right of the spacer gel (S.g.) in Fig. 18) as well as those still present in the sample (Sa.g.) and spacer (S.g.) gels were laid along one side of glass slides (1 gel column per slide) and cooled agar was poured onto each slide around the electrophoresed gel. Upon solidification of the agar, 2 thin strips of filter paper were soaked with antiserum and were placed, one atop the other, on the agar, parallel to and 5 mm away from the gel column. At the end of 3-5 days the filter paper strips were removed and the precipitin arcs which had formed were recorded as described above. In Fig. 18, the filter paper strips have been removed and the 2 slides placed side-by-side. The sample gel (Sa.g.) was lost from the upper gel column after the reactions were completed. The dark strips over the gels to the right of the spacer gels (S.g.) are artifacts.

Foot muscle extracts used in the immunoelectrophoretic tests were from Pu Yen *O. h. formosana* and *O. h. nosophora*. The antiserum was the same (5th day anti-Pu Yen serum) as that used in the specific absorption tests.

3. Results

As shown in Fig. 18, the sources of antigens operative in specific absorption tests were fractions of a slowly migrating nature of high molecular weight. The

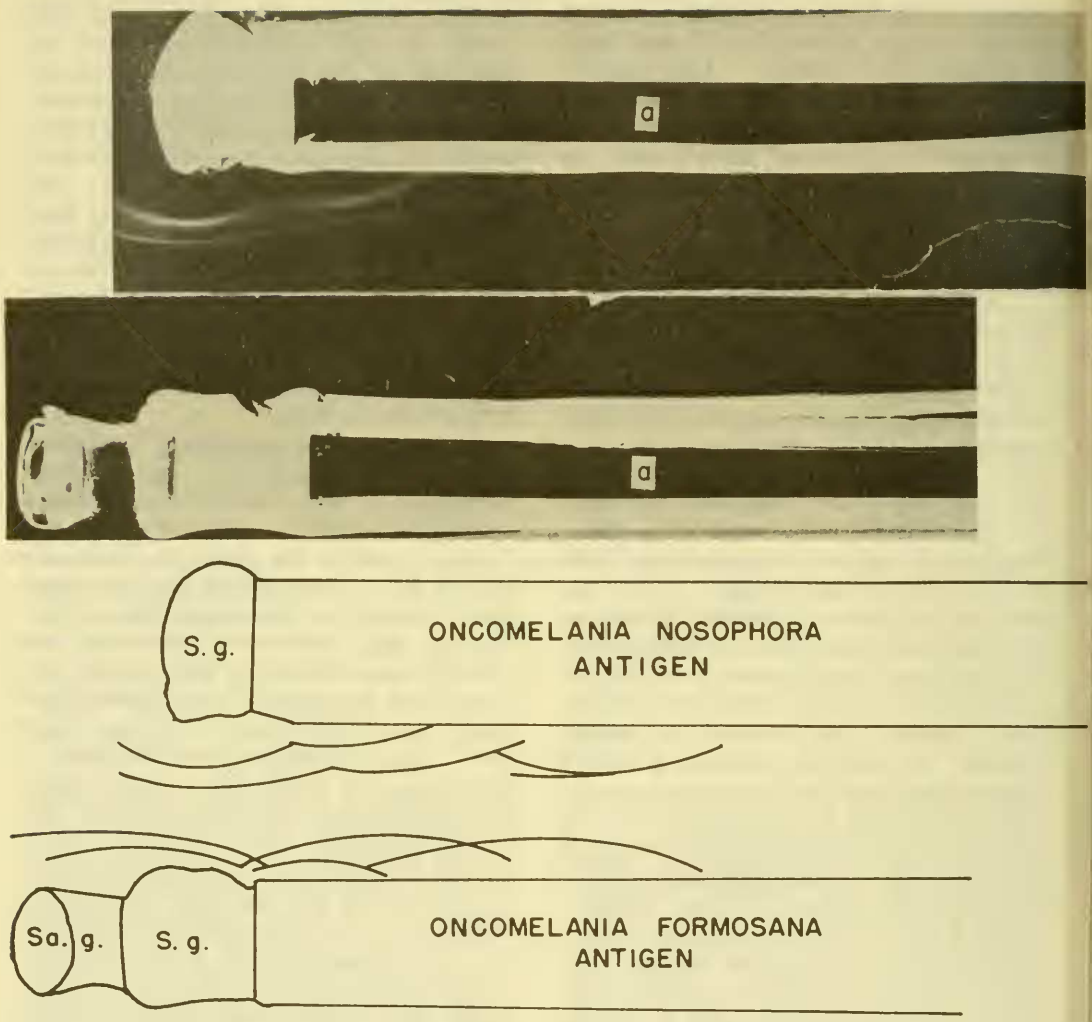
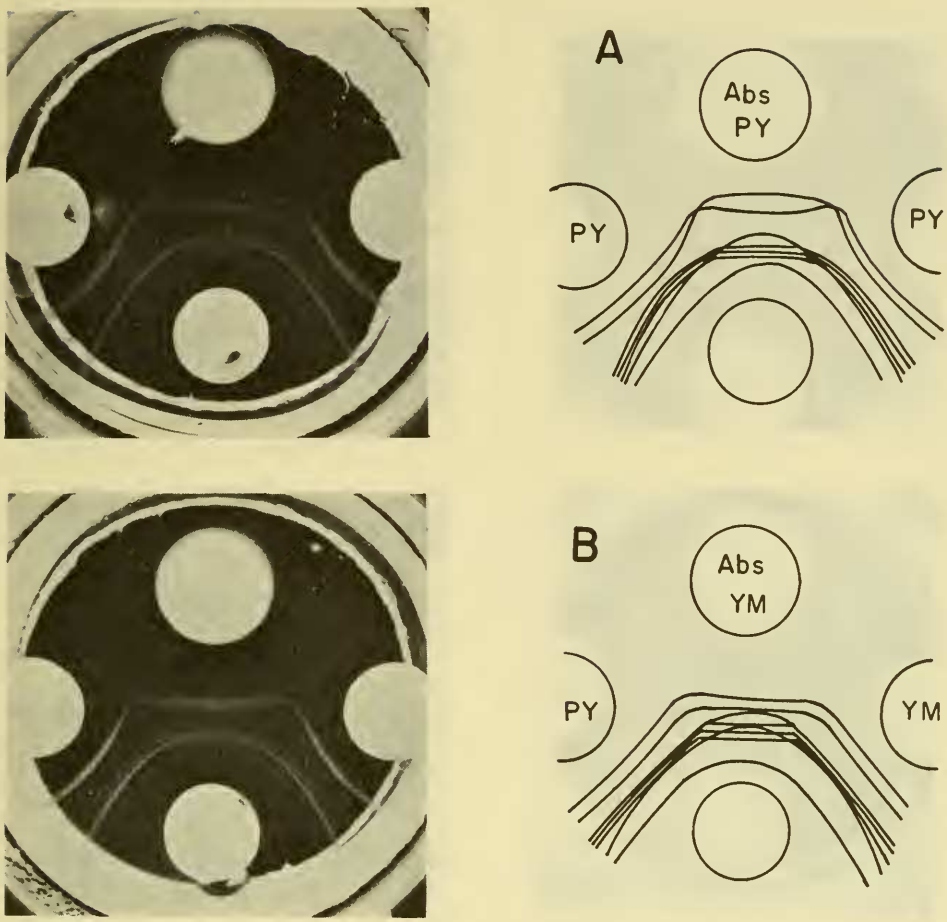


FIG. 18. Immunoelectrophoretic precipitin patterns obtained on slides with filter paper strips soaked in 5th day anti-Pu Yen serum and 2 gel columns of electrophoretically separated proteins from snail foot muscle extract. The filter paper strips have been removed; the sample gel (Sa.g.) of the upper gel column was lost prior to photographing the results. Prints of 2 slide set-ups were placed together. The antigen-antibody interference arcs, clear in the gel, did not reproduce well in the lower photographic print. The dark strips (a) over the gel columns are artifacts of reproduction occurring where the surface of the gel columns emerged above the enveloping layer of agar. The interference arcs showed visible reactions only up to component 14 (see Fig. 17). Several strongly reacting systems involved antigens present in the sample (Sa.g.) and spacer (S.g.) gels that were not part of the electrophoretically separated components.

antigens farthest from the origin of the gel column correspond to band 15 in Fig. 16B. Several antigens never enter the protein separating gel (right side, Fig. 18), but remain in the sample gel (Sa.g.) or spacer gel (s.g.).

From the long precipitin arcs showing no spurs but a slight dip (or indentation) one would assume that several separated fractions had homologous antigenicity (Fig. 18).

In the specific absorption tests, the



FIGS. 19-25. Photographs and tracings of immunodiffusion precipitin patterns using a technique of specific absorption. The homologous reactions involved *Oncomelania hupensis formosana* Pu Yen antigens (PY) (in one of the lateral wells) and 5th or 10th day anti-Pu Yen serum (top and bottom wells). Heterologous antigens (the other lateral well) were from the following: Yueh Mei (YM) and I-lan populations of *O. h. formosana; O. h. chiui* (TC); *O. h. hupensis* (OH); *O. h. nosophora* (ON); and *O. h. quadrasi* (OQ). "Abs" indicates antiserum absorbed by antigens as marked in the "Abs" well. Note opaque precipitates enlarging that well. The homologous unabsorbed antiserum was placed in the unmarked (lower) wells. The centers of the wells are 12 and 16 mm apart.

FIG. 19. Specific ansorption with homologous Pu Yen antigens (PY) in A and heterologous Yueh Mei antigens (YM) in B, using 5th day anti-Pu Yen serum, yields homologous results.

results shown in Figs. 19-23 were derived from antiserum obtained 5 days after the last injection, and those shown in Figs. 24-25 from 10th day antiserum. The latter show patterns with fewer and slightly weaker precipitation systems.

In the control (homologous or reference) reactions (anti-Pu Yen serum against Pu Yen snail foot tissue extract) the number of visable systems varied from 6-10.

As shown in Figs. 19-22, antigen-

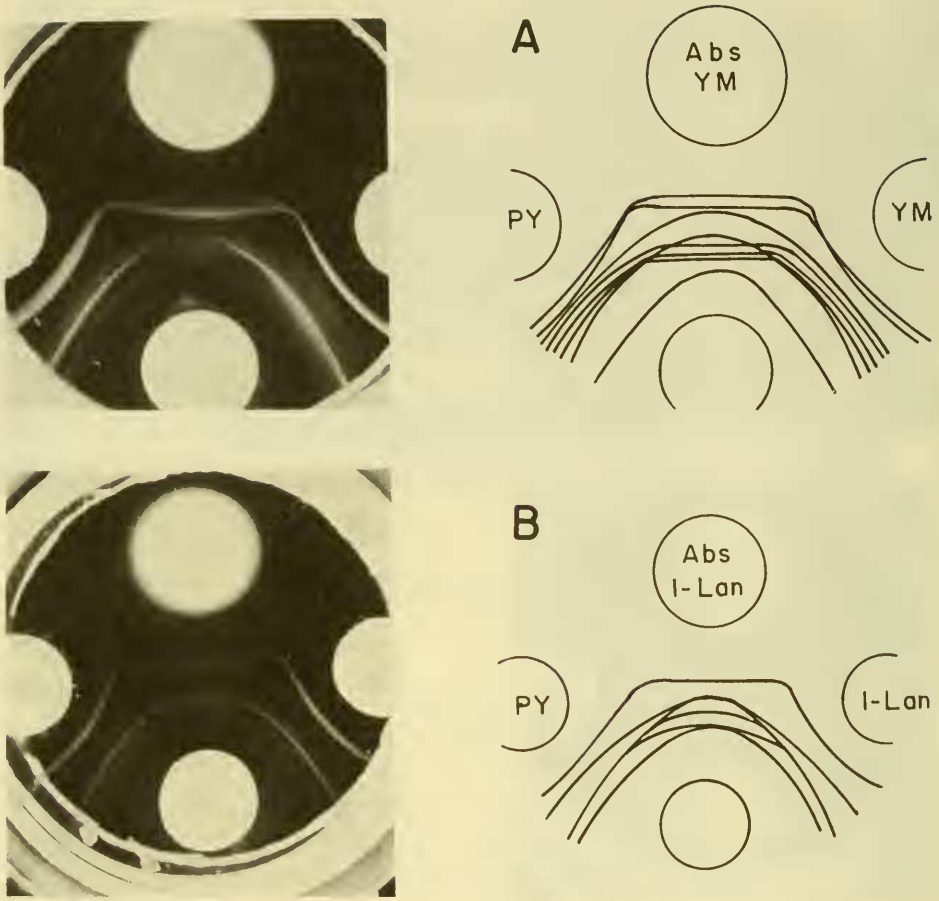
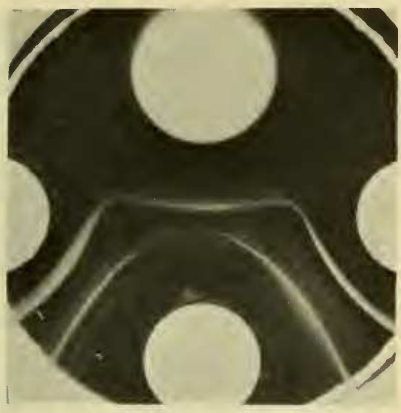


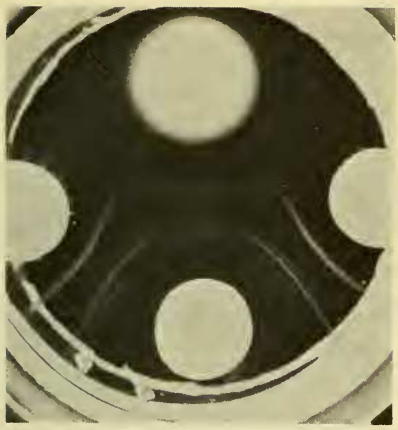
FIG. 20. Results with the heterologous Yueh Mei (A) and I-lan (B) populations of *Oncomelania hupensis formosana*, using 5th day anti-Pu Yen serum, show homologous reactions.

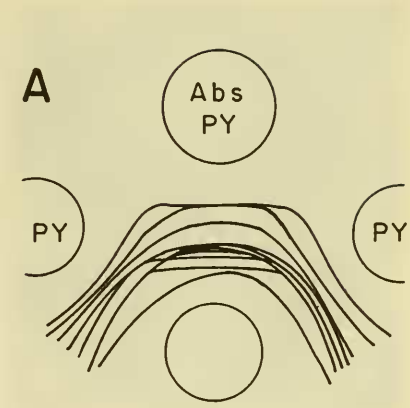
antibody reactions were those of identity (i.e., type 1 in system of reaction classification reviewed by Ouchterlony, 1958). The symmetry of reaction indicated that the initial concentration of antigens was similar in each of the 2 lateral wells. Homologous results were obtained with *Oncomelania hupensis formosana* from Pu Yen (control reaction; Figs. 19A, 21A), Yueh Mei (Figs. 19B, 20A) and I-lan (Figs. 20B, 22A) as well as with *O. h. chiui* (Figs. 21B, 22B). In one experiment (Figs. 22A), the reactions were not strong and one interference arc did not come to completion.

When the other subspecies of *Oncomelania* were tested it appeared that 20-40% of the interference arc systems demonstrated in *O. h. formosana* and *O. h. chiui* were not present (Table 14). As seen in Fig. 23, there were 3 distinct precipitin systems particular to *O. h. formosana* and not present, or of too low a concentration to be demonstrated, in *O. h. hupensis*. The reactions are type 3 (partial identity) in Ouchterlony's system of classification.

The reactions obtained with 10th day antiserum (Figs. 24-25) were relatively weaker, but clearly showed that *O. h. quadrasi* definitely lacked 1-2 systems that are characteristic for *O. h. for-*







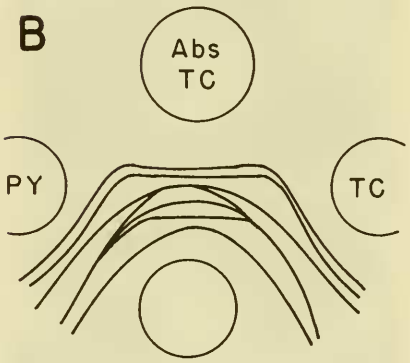


FIG. 21. Results with homologous antigens (PY) in A and with heterologous *Oncomelania hupensis chiui* antigens (TC) in B, using 5th day anti-Pu Yen serum, show no antigenic differences between the 2 taxa.

mosana, and had a reaction of both type 2 (non-identity) and type 3 (partial identity). *O. h. nosophora* equally lacked 2 systems particular to *O. h. formosana* and showed the same reaction types as *O. h. quadrasi*.

4. Discussion

Individual antigen-antibody systems serve as valid characters for a certain level of systematic comparison; it is therefore evident that, using these characters, *Oncomelania hupensis chiui* and populations of *O. h. formosana* are at the same taxon level. In terms of these immuno-diffusion systems *O. h. formosana* and *O. h. chiui* form a grouping which, on account of their antigenic properties, excludes the other subspecies of *O. hupensis*.

It is not intended here to rank the degree of difference existing in these other subspecies, but only to point out that differences do occur. Suffice it to note that the differences observed between *Oncomelania hupensis formosana*

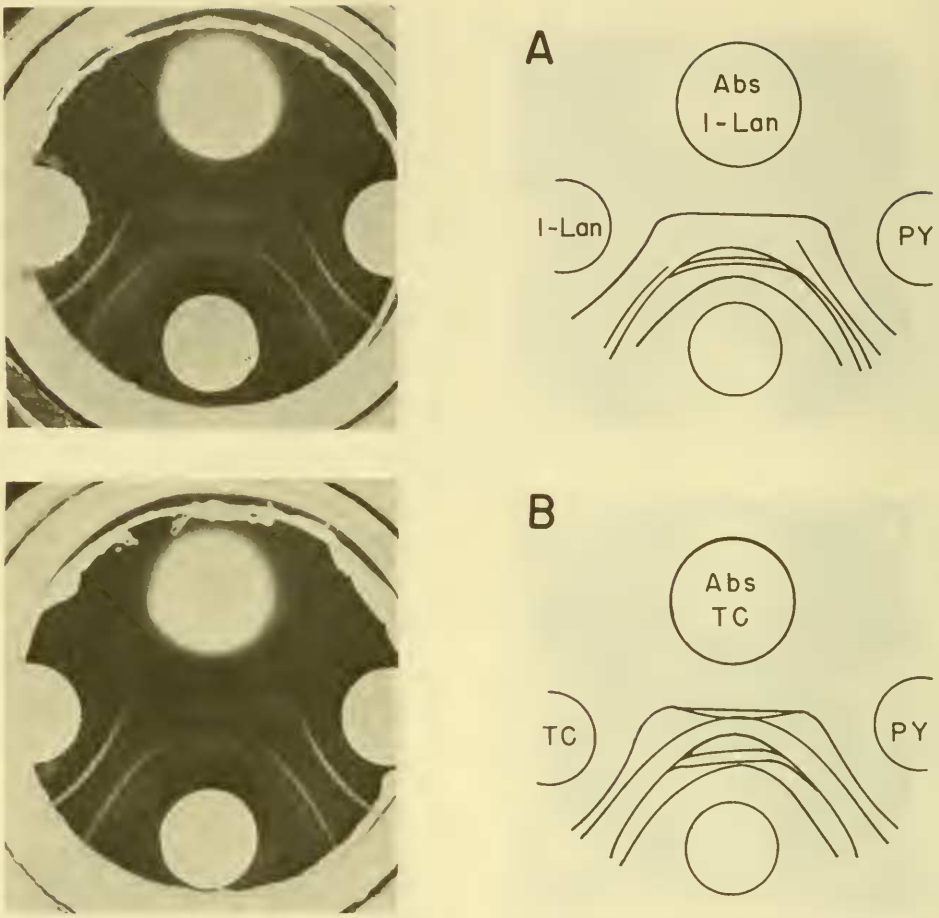


FIG. 22. Results with heterologous antigens from the I-lan populations of *Oncomelania hupensis formosana* (A) and *O. h. chiui* (B), using 5th day anti-Pu Yen serum, show no antigenic differences between the 2 taxa. In A, 1 weak precipitin arc did not completely develop in this particular reaction.

on the one hand, and *O. h. nosophora* and *O. h. quadrasi* on the other, involve the larger molecular components from the PY well (Figs. 24-25), which are slow in diffusion, while those seen in *O. h. hupensis* partly involve some of the smaller molecular weight, more rapidly diffusing components (Fig. 23), which have migrated farther from the PY well. However, differences presumably arising from the use of 5th day antiserum in the former 2 and of 10th day antiserum in the latter subspecies should not be disregarded.

In general, on strength of reaction, it appeared that *Oncomelania hupensis quadrasi* was more closely allied to *O. h. formosana* than was either *O. h. nosophora* or *O. h. hupensis* (Figs. 23-25).

The lack of demonstrable antigen-antibody systems in the fast moving fractions of the immunoelectrophoretic tests, (i.e., those from component 16 to the

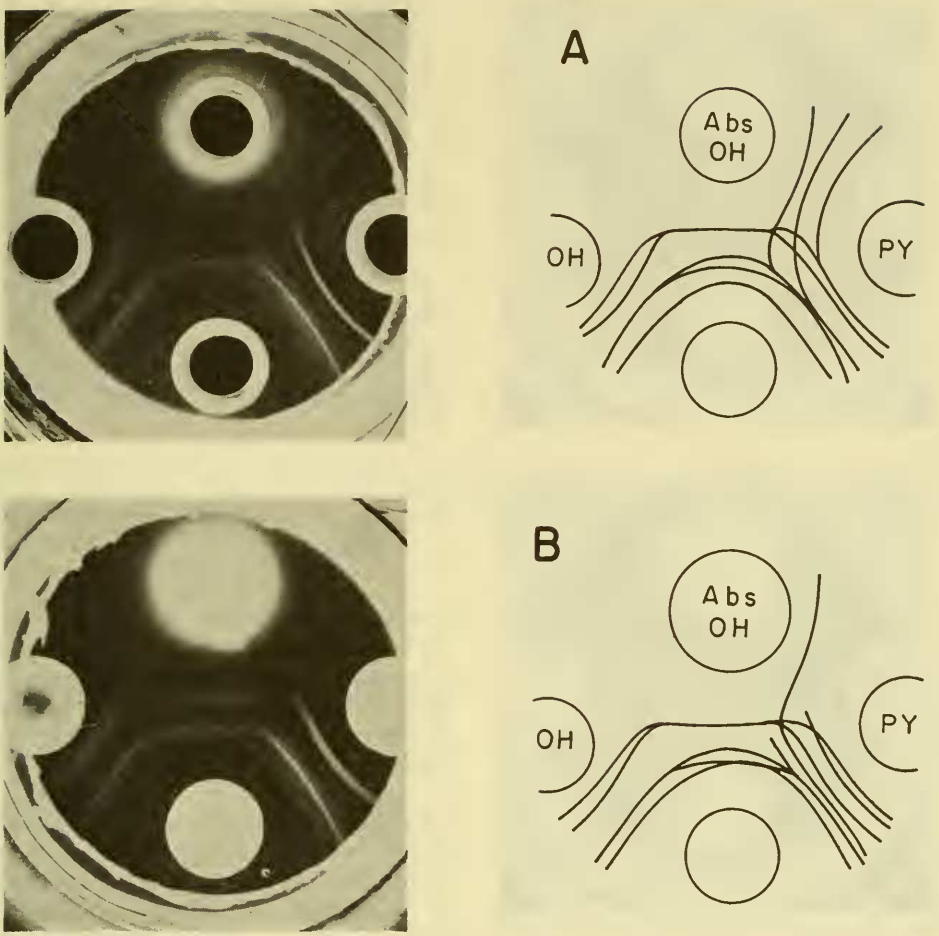


FIG. 23. Specific absorptions (A and B) both involving *Oncomelania hupensis hupensis* (OH) and Pu Yen *O. h. formosana* (PY) antigens, and 5th day anti-Pu Yen serum: 3 precipitin systems are particular to Pu Yen snails (PY).

front) was possibly due to so low a concentration of the reactants in those fractions, that visible precipitation did not occur. Other possible reasons are low concentration of those fractions in the injection series and/or poor antigenicity.

CONCLUDING DISCUSSION

Malacological

It was necessary to use an integrated approach in order to assign the taxon here investigated to each of the various category levels from subfamily to sub specific.

Characters of pedal crease, head morphology, mode of progression, ecology, egg laying, configuration of the central tooth of the radula, and general anatomy all indicated that the snail in question belonged in the hydrobiid subfamily Pomatiopsinae (Stimpson, 1865; Davis, 1966, 1967) of which *Oncomelania*, but not *Tricula*, is a member.

The genus *Tricula* (Hydrobiidae, Triculinae) is distinctly different from

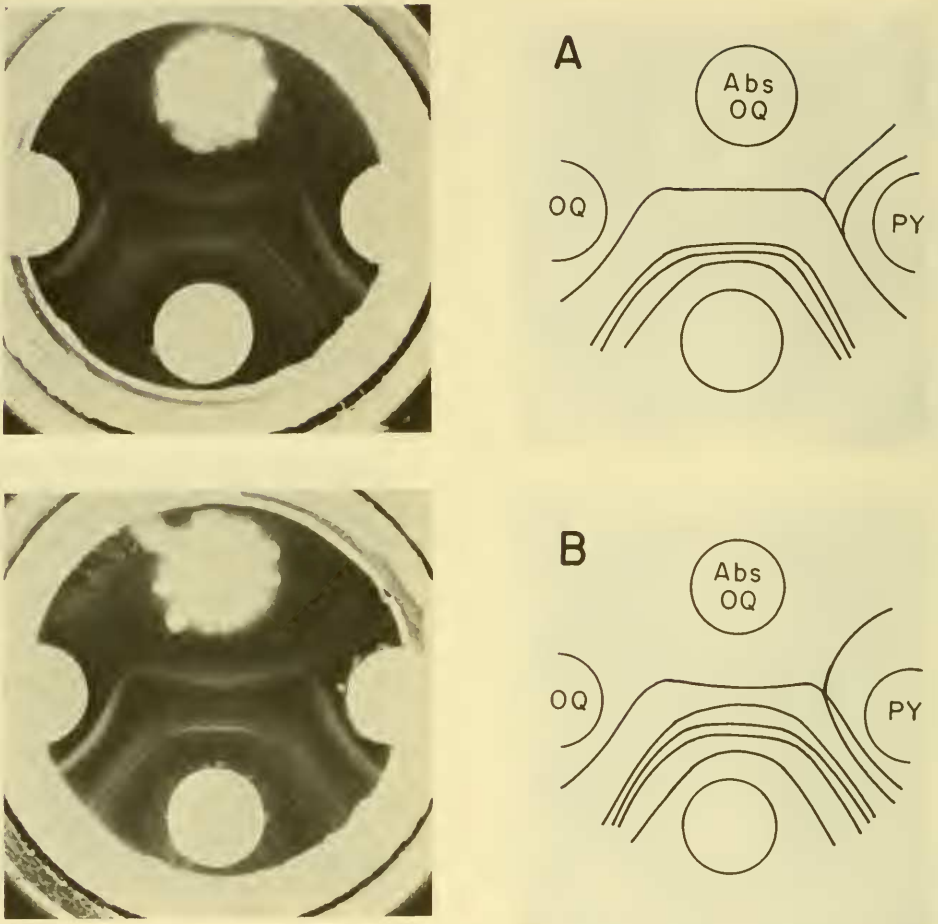
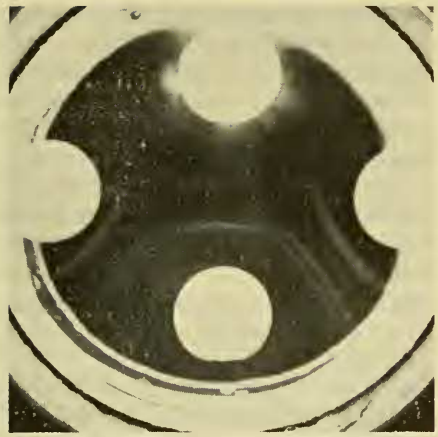


FIG. 24. Specific absorptions (A and B) both involving *Oncomelania hupensis quadrasi* (OQ) and Pu Yen *O. h. formosana* (PY) antigens, and 10th day anti-Pu Yen serum: 1-2 precipitin systems, quite weak, are particular to the Pu Yen snails (PY).

Oncomelania. Even on the basis of classical conchology the snail could not be assigned to the former genus. I have studied the types of *Tricula* from India and the Shan States of Burma which are stored at the Zoological Survey of India, Calcutta. The type of genus, *Tricula montana* Benson, was discovered by me in 1964 in the Goodwin-Austin collection at the British Museum. A vial containing 2 specimens constituted the type series. I chose one as lectotype, the other as paralectotype. The lectotype was given the accession number 1964426, the paralectotype 1964427.

On the basis of shell, species of *Tricula* vary from very small (2.4 mm, *T. gravelyi*) to moderately large (6.0 mm, *T. taylori*). On the whole they are small (*T. montana*, 3.5-3.8 mm; *T. gregoriana expansa*, 5.0 mm). By contrast the taxa included in *Oncomelania* range from 4.7-10.0 mm.

Species of *Tricula* are characteris-



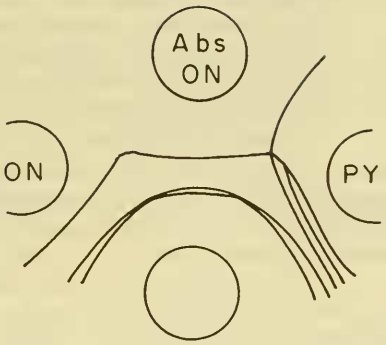


FIG. 25. Specific absorption involving *Oncomelania hupensis nosophora* (ON) and Pu Yen *O. h. formosana* antigens, using 10th day anti-Pu Yen serum. One weak system was particular to the Pu Yen snails (PY).

tically glossy or white-polished when cleaned, contrasting with the opaque to translucent yellowish shell of *Oncomelania*. They lack the varix and wide umbilicus of *Oncomelania*. Most species of *Tricula* are without umbilicus or have only a slight umbilical slit.

Several species of *Tricula* are characterized by very flat-sided whorls (*T. taylori*) and have a keel at the periphery (*T. horae*, *T. horae* v. *major*), conditions not found in *Oncomelania*.

As pointed out by Rao (1928), the anterior margin of the aperture may project "considerably in advance of the outline of the last whorl" (apertural view) as in *Tricula montana*. In *Oncomelania* the anterior margin of the aperture grows in regular even symmetry with preceding whorls.

Finally, in *Tricula* the adapical end of the aperture is quite narrowed and somewhat produced (*T. montana*, *T. martini*), and the aperture is narrowly ovate to pyriform. These features contrast with the widely ovate aperture in *Oncomelania* where the adapical portion is only slightly constricted.

Habe & Miyazaki (1962) stated that the snail here identified as *Oncomelania hupensis chiui*, was an "ally" to "*Katayama formosana* (Pilsbry et Hirase)" (i.e., *Oncomelania hupensis formosana*) but that the latter "differs from the new species in having the elongate oval shell with acute apex and yellowish brown color." Actually, as shown in the discussion on the shell, when this snail is bred under optimal conditions in the laboratory, it does have the characteristics quoted for *Oncomelania hupensis formosana*. What one observes in nature is an ecophenotype in which the nature of the environment causes extreme erosion and wear on the shell to the extent that the thickened outer lip does not develop and shells appear very short and globose.

A parallel case is seen in the habitat of *Tricula minima* (= *Fukuia kikuchi* Kuroda) on Sado Island off Honshu, Japan. Snails similarly living on a steep gradient in trickling water also had extremely

TABLE 14. Proportion of congruent antigen-antibody systems in various subspecies of *Oncomelania hupensis* as compared to those found in *O. h. formosana* from the Pu Yen area by immuno-diffusion, using the specific absorption technique (compare with Figs. 19-25)

Subspecies of *Oncomelania hupensis*	Area of origin	Precipitin systems found %
	Taiwan*	
formosana	Pu Yen	100
formosana	Yueh Mei	100
formosana	I-lan	100
chiui	A-li-lao	100
quadrasi	Philippines**	66-83
hupensis	China**	63
nosophora	Japan**	60

* Compare with Fig. 1.

** from field collected or laboratory stocks of snails from these countries.

eroded shells, appeared badly worn, and were short and globose.

The internal anatomy of *Oncomelania hupensis chiui* is strikingly similar to that of *Oncomelania hupensis formosana*. Therefore, on the basis of both shell and anatomy, the taxon is assigned to the genus *Oncomelania*. Creation of a separate genus is certainly not warranted nor should it even be considered. Only much larger anatomical differences would justify such a decision. Examples of such differences are given by Davis (1967), who discusses separate generic status for *Oncomelania* and *Pomatiopsis* (the latter genus being the one most closely related to *Oncomelania*). The anatomy of *Tricula* (Davis, MS) shows major important differences, which places the genus in the hydrobiid subfamily Triculinae.

There are numerous detailed anatomical similarities of structure between the snail discussed and *Oncomelania hupensis formosana* and 5-6 more important differences. The smaller size, reduced number of gill lamellae, slight varix, greater size of certain nervous structures, and differences in frequency of certain denticular counts on the radula are, however, not considered sufficient to justify full specific status, for the following reasons: 1) The above mentioned overwhelming number of similar structures and substructures between *O. h. chiui* and *O. h. formosana*. 2) The complete homogeneity in the characters of antigen-antibody systems between the snail in question and different populations of *O. h. formosana*, even though homogeneity was not as complete for the other subspecies of *Oncomelania hupensis*. When all subspecies were tested in a similar manner, it was apparent that *O. h. formosana* had unique systems either not present in the other subspecies, or of very low density. 3) *O. h. chiui* will mate with *O. h. formosana* and produce fertile hybrids. The production rate for young (hybrids) is the same as that for *O. h. formosana*. 4) The electrophoretic densitometric pattern of *O. h. chiui* clearly fits the pattern for I-lan *O. h. formosana;* in particular it closely resembled the church population. The resemblance in patterns is decisive, particularly as *O. h. formosana* from Pu Yen had a different densitometric pattern indicative of a definite population difference.

For these various reasons the taxon is assigned subspecific rank under *Oncomelania hupensis*. On the basis of electrophoretic pattern it would appear that *O. h. chiui* owed its origin to that stock which also gave rise to present day I-lan snails. As seen in Fig. 1, the localities of A-li-lao and I-lan are rather close to one another, in comparison to the Pu Yen and Yueh Mei

areas; however, they are separated by extremely high and rugged mountains which rise rather abruptly from the sea. I-lan county is, itself, quite an isolated pocket.

Taiwan is characterized by such isolated habitats which provide the segregation necessary for conservation of local genetic change and eventual speciation.

Parasitological

After Dr. Chiu and I had initially recognized that *"Tricula chiui"* closely resembled *Oncomelania*, the snail intermediate host of *Schistosoma japonicum* in the Far East, this relationship was further confirmed parasitologically by successful infection of that snail with the Formosan, solely zoophylic strain of *S. japonicum* (Chiu, 1965b), which is endemic in central western Taiwan, Chang-hua county.

Moreover, the anatomical and biophysical studies described in this paper have not only indicated the snail first described as *"Tricula"* to be a subspecies of *Oncomelania hupensis*, but to be most closely related to *O. h. formosana*, and, in particular, to a population of that subspecies from I-lan county, also located in the northern tip of Taiwan.

The first indication of susceptibility of Formosan *Oncomelania hupensis formosana* to human schistosomes was given by Moose & Williams (1964a, b), who succeeded in infecting I-lan snails with schistosome strains from the Philippines and Japan. These findings suggested that *O. h. chiui* might also be susceptible to infection with human strains of *S. japonicum*. Chiu (1967) has indeed shown that the snail can be infected with the Japanese strain of the parasite.

These recent discoveries are of considerable epidemiological interest. No longer does Hsü & Hsü's (1962) statement hold that: "since oncomelanian snails in Formosa are not susceptible to imported strains from Japan or the Chinese mainland, this may be the reason - a fortunate reason - why the human strain was not able to establish and complete its life cycle on Formosa." There is now no doubt, as Moose & Williams (1964b) stated, that "oncomelanid snails susceptible to infection with human strains of *Schistosoma japonicum* do occur on the island of Taiwan."

Recent investigations of the diverse susceptibility of geographical races of *Oncomelania hupensis formosana* to various schistosome strains (Moose & Williams, 1963, 1964b) reveal that the greatest complexity of snail parasite-relations is to be found on Taiwan. The distinct subunits of *Oncomelania* are just beginning to be studied in relation to presence, complexity and relationships. They vary in terms of slightly different ecology, somewhat different general appearance, and vastly different susceptibilities. In the light of these fairly new epidemiological findings and the biological data presented here, it appears that Taiwan provides the main focal point for studying and establishing the underlying causes for the complex shifts in genetic potential discussed in the literature as "strains" of *S. japonicum*. Likewise to be pointed out is the fact that data derived from malacological studies are of value in predicting, to a degree, the relationship of a snail taxon and its susceptibility to parasitic involvement.

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RÉSUMÉ

UNE ETUDE SYSTEMATIQUE D'*ONCOMELANIA HUPENSIS* CHIUI (GASTROPODA: HYDROBIIDAE)

G. M. Davis

Le gastropode Hydrobiidae que Chiu (1961) signala être le premier hôte de *Paragonimus iloktsuenensis* à Formose fut décrit comme *Tricula chiui* par Habe et Miyazaki (1962). Le gastropode, trouvé dans un bassin isolé du Nord de Formose (région d'A-li-lao), se révéla plus tard susceptible de transmettre aussi *Schistosoma japonicum* (Chiu, 1965b, 1967). Des observations anatomiques ont désormais montré que ce gastropode appartient au genre *Oncomelania*. Il est très proche des sous-espèces de *O. hupensis*, décrites sour les noms de *O. h. formosana* (Davis, 1967), *O. h. nosophora* et *O. h. quadrasi* (Davis, non publié), parceque: 1) la verge porte une papille et des bandes cilées caractéristiques près de la pointe; 2) la vésicule séminale forme un "noeud"; 3) chez la femelle, le canal de la spermathèque et le "canal du sperme" apparaissent sur le côté droit de la poche copulatrice, près de l'extrémité antérieure, comme deux tubes séparés mais entourés d'une enveloppe commune de tissu conjonctif; 4) l'oviducte s'enroule autour du réceptacle séminal de façon distincte; 5) la structure des glandes mâle et femelle est semblable; 6) la coquille est typiquement d'un *Oncomelania* malgré la varice (bourrelet creux) peu marquée.

Des études d'immunologie, utilisant la méthode d'Ouchterlony avec double diffusion d'antiserum (anti-muscle pédieux d'*O. h. formosana*), ont indiqué que *"Tricula chiui"* est plus proche de *O. h. formosana* que de toute autre sous-espèce de *Oncomelania hupensis*, car: 1) tous les systèmes antigene-anticorps sont homologues entre eux; 2) ils ont des systèmes qui ne sont pas présents chez les autres sous-espèces de *O. hupensis* ou qui n'y sont que partiellement identiques.

L'électrophorèse sur polyacrylamide des protéïnes extraites du muscle pédieux de *"T. chiui"* et de plusieurs populations de *O. h. formosana*, ont montré que la courbe de densité optique de *"T. chiui"* est plus proche de celle d'une population de *O. h. formosana* du Nord-Est de Formose (région d'I-lan), que d'aucune autre.

Des études d'hybridation ont montré que *"T. chiui"* produit des hybrides fertiles avec *O. h. formosana*.

En tenant compte de ces observations il apparait évident que le gastropode originellement nommé *"Tricula chiui"* a tiré son origine d'un stock de *O. h. formosana* normal de la région d'I-lan et qu'à la suite de son isolement en bordure de mer à A-li-lao, que de hautes montagnes séparent de la région d'I-lan, ce stock a acquis de nouvelles caractéristiques qui justifient le statut de sous-espèce, à savoir, 1) une coquille plus courte, 2) un nombre de filaments branchiaux significativement plus faible que chez les autres espèces de *O. hupensis*, 3) une coquille avec une varice obsolète, 4) un connectif pleurosupraoesophagien plus long que chez *O. h. formosana*, 5) un plus grand ganglion supraoesophagien, 6) une différence dans la fréquence du nombre de cuspides sur les dents de la radula. En conséquence le taxon est nommé *Oncomelania hupensis chiui*.

RESUMEN

ESTUDIO SISTEMATICO DE *ONCOMELANIA HUPENSIS CHIUI* (GASTROPODA: HYDROBIIDAE)

G. M. Davis

El caracol hidróbido que Chiu (1961) registró como el primer huéspued intermediario de *Paragonimus iloktsuenensis* en Taiwan (Formosa) fué descripto como *Tricula chiui* por Habe & Miyazaki (1962). Este caracol, encontrado en una cuenca aislada del norte de Taiwan (Ai-li-lao area), posteriormente demostró ser susceptible a *Schistosoma japonicum* (Chiu 1965b, 1967). Sus detalles anatómicos muestran ahora que pertenece al género *Oncomelania*. Es muy afín a la subespeciesde *O. hupensis*, descriptas como *O. h. formosana* (Davis, 1967), *O. h. nosophora*, y *O. h. quadrasi* (Davis, sin publicar), porque: 1) la verga tiene papila y caracteristica banda ciliar cerca de la punta; 2) la vesícula seminal es nudosa; 3) la espermateca y ducto espermático salen del borde lateral derecho de la bolsa copulatriz cerca del extremo anterior, como un par de tubos separados pero envueltos en una vaina común de tejido conjuntivo; 4) el oviducto se arrolla sobre el recepetaculo seminal en una forma particular; 5) la estructura de las gonadas maculina y femenina son similares; 6) la concha es distintamente del tipo de *Oncomelania* a pesar del poco desarrollo de la várice.

Estudios immunológicos usando micro-Ouchterlong en pruebas de doble difusión antisuero (anti- *O. h. formosana*, extrato del musculo pedal), indicaron que *"Tricula chiui"* estaba asociada más estrechamente con poblaciones de *O. h. formosana* que con las otras subespecies de *Oncomelania hupensis* porque: 1) todos los sistemas de antigenos-anticuerpos eran homólogos entre ellos; 2) tenian sistemas no presentes, o sólo parcialmente iguales, con aquellos que aparecen en otras subespecies de *O. hupensis*.

Electroforesis poliacrimalida de proteinas extractadas del musculo pedal de *"T. chiui"* y varias poblaciones de *O. h. formosana* mostró que la primera tiene un perfil densitométrico de los componentes proteicos separados, más similar a una población de *O. h. formosana* del N. E. de Taiwan (I-lan distrito) que a cualquier otra.

Estudios de hybridización mostraron que *"T. chiui"* produjeron híbridos fertiles con *O. h. formosana*.

Por la reunión de estos datos, parece como muy probable que el caracol originalmente llamado *"Tricula chiui"* tuvo su origen en el linaje que dió lugar al surgimiento de la corriente *O. h. formosana* de I-lan, y que el aislamiento que siguió cerca del borde maritimo en A-li-lao, donde altas montañas separa esta region de la I-lan, produjo caracteristicas que justifican el status subespecifico, como ser, 1) conchilla mucho más corta, 2) significativamente menor número de filamentos branquiales que en las otras subespecies de *O. hupensis*, 3) conchilla con várice rudimentaria, 4) un conectivo pleuro-supraefágico más largo que en *O. h. formosana*, 5) ganglio supraesofágico más grande, 6) diferencia en frecuencia de número de cúspides en los varios dientes radulares. A acuerdo con todo esto el taxon debe llamarse *Oncomelania hupensis chiui*.

АБСТРАКТ

ИЗУЧЕНИЕ СИСТЕМАТИКИ *ONCOMELANIA HUPENSIS CHIUI* (GASTROPODA: HYDROBIIDAE)

Г. М. ДЕВИС

Гидробииды, которые по Чиу (1961) являются первыми промежуточными хозяевами *Paragonimus iloktsuensis* на Тайване, были описаны Хабе и Миязаки (1962) как *Tricula chiui*. Эти моллюски, найденные в изолированном бассейне на Северном Тайване (район Аи-ли-ляо), как было установлено позже, также восприимчивы к *Schistosoma japonicum* (Чиу, 1965б, 1967).

Судя по анатомическим данным, было выяснено, что этот моллюск относится к *Oncomelania*. Он близко родственен к подвиду *O. hupensis*, как это было описано для *O. h. formosana* (Дэвис, 1967), для *O. h. nosophora* и *O. h. quadrasi* ((Дэвис, неопубликовано), потому что: 1) по краю имеются папиллы и характерная кайма ресничек у верхушки; 2) семенной пузырек узловатый; 3) сперматеки и семепротоки отходят от правого переднего бокового края совокупительной сумки ввиде двух отдельных трубок, связанных между собой общей соединительнотканной оболочкой; 4) яйуепровод образует характерный изгиб над семенным пузырьком; 5) строение мужской и женской гонад сходно; 6) раковина типа *Oncomelania*, несмотря на отсутствие varix.

Иммунологическое исследование при помощи тестов двойной диффузии методом микро-Аухтерлонга с адсорбцией антисерума (как анти - употреблялся экстракт из ножного мускула *Oncomelania hupensis formosana*), показало, что *"Tricula chiui"* стоит ближе к популяциям *O. h. formosana*, чем к другим подвидам *O. hupensis*, потому, что: 1) все системы антиген-антител гомологичны между собой; 2) имеются системы, не представленные или лишв частично идентичные тем, которые встречаются у других подвидов *O. hupensis*.

Полиакриламидный электрофорез белков, экстрагированных из ножного мускула *"Tricula chiui"* и некоторых популяций *Oncomelania hupensis formosana* показало, что первая имела денситометрические профили отдельных компонентовв белков более сходные 1 популяции *O. h. formosana* из северо-восточного Тайваня (район Ай-лян), чем к каким-нибудь другим.

Гибродизация показала, что *"Tricula chiui"* давала с *Oncomelania hupensis formosana* плодовитое потомство.

Из всех этих данных следует, что моллюски, первоначально названные *"Tricula chiui"* вероятнее всего произошли от *Oncomelania hupensis formosana* из района Ай-лян; последующая изоляция у берега моря в районе Ай-ли-

ляо, где высокие горы отделяют их от района Ай-лян, привела к тому, что эта популяция приобрела черты, придавшие им ранг подвида, а именно: 1) гораздо более укороченная раковина; 2) значительно меньшее количество жаберных нитей, чем у других подвидов *O. hupensis* ; 3) раковина без varix; 4) плевро-надглоточная коннектива более длинная, чем это имеется у *O. h. formosana* ; 5) более крупный надглоточный ганглий; 6) различия в частоте и количестве зубцов на различных зубах радулы.

В соответствии с этим данная форма получила название *Oncomelania hupensis chiui*.